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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

SIXTEENTH ANNUAL MEETING.

PHILADELPHIA, PA., DECEMBER 29 and 30, 1903.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

THE SURVIVAL OF IRRITABILITY IN MAMMALIAN NERVES AFTER REMOVAL FROM THE BODY.

By W. D. CUTTER and P. K. GILMAN.

MAKING use of the fact noted by other observers (Bernard, Schiff, Israel, Greene), that the mammalian nerve retains its irritability for some time after removal from the body, the authors attempted to determine the duration of this survival, the variations in irritability during the period of survival, and, lastly, the effect of prolonged anæsthesia upon the phenomenon. Irritability was determined by measuring the action current of the nerve when stimulated by a series of induction shocks. The experiments were made upon dogs, and the sciatics of both legs were taken for observation. One sciatic was removed as soon as the animal was anæsthetized sufficiently for the operation. The nerve was placed at once in the moist chamber, and its action current was determined at intervals of half an hour, as long as a response could be obtained to stimulation. With the values of these action currents as ordinates, a curve was constructed, showing the duration and variations of irritability in the "unanæsthetized nerve" during the period of observation. The other sciatic was left in the animal for a period of four to six hours, and during this time the animal was kept completely anæsthetized by morphia and ether. At the end of this period, there was a considerable fall in rectal temperature (30°–31° C.). The "anæsthetized" nerve was then removed, and galvanometric observations were made similar to those just described. The results obtained show that the nerve removed from the anæsthetized (and cooled) animal survives for a longer period than that taken from the animal at the beginning of the period of anæsthesia, the difference in time of survival being as

much as four or five hours. A more marked difference, however, is that the "anæsthetized" nerve exhibits throughout a much greater irritability. The curves obtained were irregular; but that for the "unanæsthetized" nerve shows a small increase in irritability occurring shortly after the excision, and soon followed by a steady decline to zero; while that for the "anæsthetized" nerve exhibits, as its most marked feature, a large and sudden increase in irritability coming on some hours after the excision, and followed by a more rapid fall to zero.

THE CONDITION OF THE VASOCONSTRICTOR NEURONS IN "SHOCK."[1]

By W. T. PORTER and W. C. QUINBY.

The normal fall of blood-pressure produced by stimuli of uniform intensity applied to the central end of the depressor nerve was measured in the rabbit and the cat. In the same animals shock was then brought on, and the measurements repeated. Following are abbreviated protocols:

July 7, 1903. — Cat, anæsthetized with ether. *9.25* A.M.: Carotid blood pressure, 105 mm. Hg. *10.20* A.M.: Stimulated central end of left depressor nerve; blood-pressure fell from 80 to 45 mm. (**44**%). *10.23* A.M.: Section of spinal cord in dorsal region; blood-pressure 36 mm. *10.50 to 11.10* A.M.: Injected 75 c.c. 0.6% sodium chloride solution in jugular vein; blood-pressure 80 mm. *11.10* A.M.: Stimulated depressor; blood-pressure fell from 80 to 43 mm. (**46**%). *11.35* A.M.: Blood-pressure 30 mm.; injected 70 c.c. sodium chloride solution; blood-pressure rose to 77 mm.; stimulated depressor; blood-pressure fell from 77 mm. to 43 mm. (**44**%). *11.50* A.M.: Blood-pressure 30 mm.

September 22, 1903. — Rabbit, anæsthetized with ether; blood-pressure in left carotid, 87 mm. Hg. *9.20* A.M.: Blood-pressure 70 mm.; stimulated central end of left depressor three times; blood-pressure fell to 50 mm. (**29**%), 55 mm. (**21**%), and 52 mm. (**26**%) respectively; burned intestines and part of parietal peritoneum with nitric acid. *9.25* A.M.: Blood-pressure 40 mm.; rectal temperature 37.6°. *9.35* A.M.: Blood-pressure

[1] A note of clinical interest regarding this investigation was published in the Boston medical and surgical journal, 1903, cxlix, pp. 455–456.

rises to 60 mm., probably from stimulation of sensory nerves by the nitric acid. *11.50* A.M.: Rectal temperature 31°; blood-pressure 30 mm.; stimulated depressor; blood-pressure fell from 30 to 21 mm. (**30**%). *12* M.: Heart feeble; injected glycerine extract of suprarenal gland; blood-pressure rose to 90 mm., but sank thereafter. *12.10* P.M.: Rectal temperature 30°; blood-pressure 33 mm.; stimulated depressor; blood-pressure fell from 33 to 25 mm. (**24**%). *12.15* P.M.: Repeated injection of suprarenal extract; blood-pressure rose temporarily to 90 mm. *12.30* P.M.: Blood-pressure 72 mm.; stimulated depressor; blood-pressure fell to 55 mm. (**24**%); blood-pressure then rose to 72 mm.; repeated the stimulation; blood-pressure fell from 65 mm. to 48 mm. (**26**%).

September 24, 1903, 9 A.M. — Rabbit, anæsthetized with ether; carotid blood-pressure 80 mm. Hg. *9.10* A.M.: Both vagi cut. *9.15* A.M.: Rectal temperature 38°; blood-pressure 67 mm.; stimulated central end of depressor nerve; blood-pressure fell to 36 mm. (**46**%). *9.20* A.M.: Exposed intestines; ligated mesenteric artery; applied nitric acid to intestines; blood-pressure rises. *9.40* A.M.: Rectal temperature, 36.6°; electrical stimulation of nerves near mesenteric artery causes the blood-pressure to fall from 70 mm. to 55 mm. Hg. *3.25* P.M.: Rectal temperature 26°; no anæsthetic has been necessary for many hours; blood-pressure 53 mm.; stimulated depressor; blood-pressure fell to 30 mm. (**43**%). *4* P.M.: Blood-pressure has for some time been about 30 mm. Hg. *4.50* P.M.: Blood-pressure 40 mm.; stimulated depressor; blood-pressure fell to 22 mm. (**45**%). *5.16* P.M. (8 hours, 16 minutes after the beginning of the experiment): Rectal temperature 25°; blood-pressure 35 mm.; stimulated depressor; blood-pressure fell to 23 mm. (**34**%).

In all cases the pressures given are diastolic, recorded by a slightly damped Hürthle membrane manometer. After stimulation of the depressor, the blood-pressure regained its former level.

From these protocols it is clear (1) that the normal percentage fall in blood-pressure may be obtained by stimulating the depressor nerve during shock; (2) if during shock the blood-pressure be raised to normal values by the injection of suprarenal extract or normal saline solution, and the depressor nerve be stimulated while the pressure is still high, the absolute fall in blood-pressure may be as great as it was in the same animal before shock began.

Exhaustion in the vasoconstrictor neurons cannot therefore be the essential cause of the symptoms termed shock.

A STUDY OF THE ERRORS INVOLVED IN THE DETERMINATION OF THE BLOOD-PRESSURES IN MAN, TOGETHER WITH A DEMONSTRATION OF THE IMPROVEMENTS IN THE SPHYGMOMANOMETER THEREBY SUGGESTED.

By JOSEPH ERLANGER.

The experiments herein recorded were performed with the object of determining the errors involved in the estimation of the maximum and minimum pressures with the sphygmomanometer devised by the author (This journal, 1902, vi, p. xxii), the particular problem being the determination of the effect of increasing the length of artery compressed.

In the first series of experiments performed, use was made of an artificial circulation scheme. These experiments showed that, as a result of putting the walls of the tubes upon the stretch, the determination made with the short tubes were higher than those made with long tubes; this effect disappeared when the length of the tube compressed exceeded 4 cm.

These experiments were repeated upon animals, the pressure being applied directly to the intact artery. The length of artery compressed (3–6 cm.) had but very little effect upon the results.

These experiments indicate that the maximum pressure, as determined with the sphygmomanometer, is the maximum lateral pressure of the artery of which the one explored is a branch. As the minimum pressure is practically the same in all of the larger arteries (Dawson), the sphygmomanometer, when applied to the brachial artery of man, determines approximately the lateral pressures — maximum and minimum — in the aorta.

In order to be able to study the effect of the tissues upon pressure determinations, estimations of pressures in dog's thighs were made with cuffs of different widths. With narrow cuffs (3.5 cm.) the resistance offered by the tissues may produce an error of 50 mm. Hg. But with broad cuffs (9 cm.) this error, under normal conditions, is probably never greater than 10 mm. Hg. The error resulting from the resistance offered by the tissues is the same for both the maximum and minimum pressures. The error that results from using the sense of touch as an indicator of the return of the pulse wave may *by chance* be balanced by the error resulting from the resistance offered by the tissues. Therefore, if an accurate method of determining the

return of the pulse wave could be adopted, the "pulse pressure" (difference between the maximum and minimum pressures) could be determined with almost absolute accuracy.

As a result of this work a broad cuff (12 cm.) has been substituted for the narrow one, and a method of determining the maximum pressure by letting the cuff "feel" the return of the pulse has been adopted. The mechanism of the instrument has been so simplified that but a single stopcock is now required.

THE RELATION BETWEEN BLOOD-PRESSURE, PULSE-PRESSURE, AND THE VELOCITY OF BLOOD-FLOW IN MAN.

By JOSEPH ERLANGER and DONALD R. HOOKER.

Experiments performed with the sphygmomanometer show that, upon changing from the recumbent to the standing posture, the minimum pressure is increased and the pulse-pressure (difference between maximum and minimum pressures) is diminished. Upon assuming the sitting posture, these pressures almost return to the values they had had while recumbent. Experiments performed with the v. Kries tachygraph show that, in agreement with the changes in pulse-pressure, the acceleration of the blood-flow per heart-beat is greatest while recumbent, and smallest while standing. Furthermore, there is an inverse relation between the pulse-rate on the one hand, and the pulse-pressure and the velocity of flow, on the other. Under perfectly normal conditions, the product of the pulse-pressure by the pulse-rate, therefore, tends to remain constant, as does the product of the acceleration by the pulse-rate. Furthermore, in any two postures, the pulse-pressures are to one another as the accelerations per pulse wave. This is in accordance with the law governing the flow of fluids through large elastic tubes.

Experiments tend to indicate that the explanation of the pressure changes that accompany changes in posture is to be found chiefly in changed hydrostatic conditions.

THE RELATION OF BLOOD-PRESSURE AND PULSE-PRESSURE TO THE SECRETION OF URINE AND TO THE SECRETION OF ALBUMIN IN A CASE OF SO-CALLED PHYSIOLOGICAL ALBUMINURIA.

By JOSEPH ERLANGER and DONALD R. HOOKER.

Experiments described in the preceding report have served the purpose of studying the relation between the "pressures" and the secretion of urine, the composition of the urine, and the secretion of albumin in a case of physiological albuminuria. In all the experiments performed, including the effects of posture, of baths in the erect posture, of muscular exertion, of meals and of daily routine, a distinct relation has been found between the magnitude of the pulse-pressure and the amounts of urine and of albumin, an increase in the pulse-pressure accompanying an increase in the amount of urine and a diminution in the amount of albumin. The rate of secretion of the urine and of albumin is apparently independent of pulse-rate and of the minimum and maximum blood-pressures; and no relation can be proved between the secretion of urine and of albumin, and the product of the pulse-rate by the pulse-pressure.

Furthermore, experiments show that posture, and hence, presumably, pulse-pressure, has a marked influence upon the composition of the urine. The amounts of the chlorides, of the phosphates, and of the total nitrogen, are much smaller in the standing than in the recumbent posture, the variations in the amounts of the phosphates and of the total nitrogen being smaller than the variations of the chlorides.

ON CO-ORDINATION OF THE VENTRICLES OF THE HEART.

By W. T. PORTER, for C. FROTHINGHAM, Jr., and W. E. LADD.

In an investigation published in this journal, 1899, vol. ii, pp. 127–136, W. T. Porter found "That any portion of the ventricle will beat synchronously with any other portion, so long as the two are connected by muscle tissue, but that synchronism immediately fails when the muscle bridge is broken, in spite of the fact that both portions may retain their normal connection with the uninjured auri-

cles." In 1899 von Vintschgau[1] observed that when a complete physiological division of the frog's ventricle is made by crushing the ventricle lengthwise with forceps until the physiological continuity of the two halves is destroyed, both halves, united only by auricular tissue, beat synchronously. This discrepancy makes it desirable (1) that the batrachian ventricle be subjected to the same method of division used by Professor Porter on the mammalian ventricle, namely, longitudinal section; (2) that Professor Porter's investigation be repeated, for his conclusion, though well supported by numerous experiments, was after all negative; and (3) that similar experiments be performed upon hearts in which the muscular connection between auricle and ventricle is less general than in the frog and tortoise, and at the same time more definite than in the mammalian heart, so that the relation of co-ordination to the muscular fibres uniting auricle and ventricle may be determined.

The present note deals with the first of these studies.

When the ventricle of the bull-frog or the tortoise heart is completely divided by an incision passing into the auricle, the separated portions may still be observed to contract synchronously, although their only muscular connection is through the auricle. Division was made in different planes, but the results were the same in all cases.

THE PASSAGE OF DIFFERENT FOOD-STUFFS FROM THE STOMACH.

By W. B. CANNON.

X-RAY shadows cast by various foods (mixed with bismuth subnitrate) afford a method of estimating the relative amount of food in the intestines at different times and in different animals. As the diameter of the intestines varies only slightly, the aggregate *length* of the shadows may be taken to indicate the amount of food. The faults of the method, due to absorption, to the intestinal loops not being parallel with the fluorescent screen, and to variations in the thickness of the food masses, are slight compared with the great differences in the amounts of the different food-stuffs in the intestine in the early stages of digestion.

In all cases the animals (cats) were given 25 c.c. of food, and the

[1] VON VINTSCHGAU: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 59.

different foods were as nearly as possible of the same consistency. Tracings of the shadows on tissue paper were made at regular intervals after feeding.

The following average figures illustrate the characteristic differences in the aggregate length (in centimetres) of the intestinal content with the various food-stuffs:

Hours after feeding	0½	1	2	3	4	5	6	7
Fats (15 cases)	5	9	14	17	16	13	13	9
Proteids (10 cases)	1	5	18	21	23	19	17	14
Carbohydrates (14 cases)	8	30	39	33	25	19	14	6

The remarkable difference between carbohydrate and proteid figures in the early stages can be accounted for by assuming that free acid is the stimulus opening the pylorus. Both carbohydrates and proteids provoke abundant gastric secretion (Pawlow). With carbohydrate food there is free acid immediately; with proteid food there is no free acid so long as acid unites with proteid. Thus the discharge of proteids would be delayed. Feeding acid proteid and also crackers wet with one per cent sodium bicarbonate solution gave further evidence favoring this theory.

Hours after feeding	0½	1	2	3	4	5	6	7
Acid proteid	6	30	43	39	28	21	14	10
Alkaline carbohydrate	0	4.5	19	28	31	29	25	21

Note that acid proteid leaves the stomach quite as rapidly as carbohydrate food, and that alkaline carbohydrate, like ordinary proteid, passes out slowly.

The addition of 0.4 per cent hydrochloric acid to carbohydrate food does not increase the rapidity of the discharge from the stomach. Hirsch and Serdjukow have noted that acid in the duodenum checks the emptying of the stomach. Observation shows no cessation of gastric peristalsis after food has passed the pylorus. The acid must therefore act on the pylorus. Tying the bile and pancreatic ducts greatly decreases the rate of discharge of carbohydrate food.

Evidence thus points to free acid in the stomach opening the pylorus, and in the duodenum closing the pylorus. But acid in

the duodenum stimulates alkaline secretions, and the acid is thereby neutralized; whereupon the acid in the stomach again opens the pylorus to allow more food to pass out. Thus proteids would be retained in the stomach until acted upon by gastric juice, and thus automatically the intestine would be guarded from being overwhelmed with food and with secretions interfering with the intestinal ferments.

THE EMPTYING OF THE HUMAN STOMACH.

By W. B. CANNON.

The usual idea of the shape and position of the stomach is taken from the figures of His and of Luschka, now copied in many text-books of anatomy. The greater curvature in these figures reaches a point considerably lower than the pylorus. Surgeons use this conception in making the gastro-enterostomy opening, for purposes of "drainage," at "the most dependent part." Conceptions of the shape and position of the stomach based on appearances in the cadaver, or in a living person relaxed in anæsthesia, may not be true for the functioning organ.

Observations with the X-rays on a normal human stomach containing food mixed with bismuth subnitrate show that while digestion is proceeding, and the stomach is emptying itself, it shortens, just as if the longitudinal and oblique fibres passing over the surfaces to the greater curvature lifted the organ up toward the one fixed point of the contracting fibres, — the cardia. Since the pylorus is also more or less fixed, it does not rise with the rest of the stomach. The consequence is that in the late stages of digestion, when the gastric contents are more fluid than in the earlier stages, the pylorus becomes the lowest point in the stomach, and the contents do not therefore have to be lifted in order to be passed out.

SUPRARENAL GRAFTING IN THE KIDNEYS OF RABBITS WITH SURVIVAL OF AN ANIMAL AFTER SUBSEQUENT REMOVAL OF THE REMAINING SUPRARENAL.

By F. C. BUSCH and C. VAN BERGEN.

The authors have made suprarenal grafts in a series of rabbits, transplanting a section of the gland into the animal's own kidney. The experiment was successful, histologically, in several animals, and

physiologically, in one. Rabbits, where total ablation of the suprarenals has been made, have invariably died from eight to ten hours after the operation. One rabbit, from whom the remaining suprarenal was removed eighty-three days after the introduction of a graft, died ten hours after the operation. The graft was found to have become entirely necrotic, and was surrounded by a thick layer of connective tissue.

In three other cases, where the animals died from ten days to two months after the introduction of the graft, either through an anæsthetic or other accident, a partial preservation of the suprarenal was found upon histological examination. In contradistinction to the results of other investigators, the authors found a survival of medullary cells and a necrosis of the cortex. This may possibly have been due to the manner in which the grafts were made.

In the single physiologically successful case (Rabbit X), at the first operation, the left suprarenal was removed *in toto*. Longisection of the gland was made on each side, and the middle half of the gland was introduced into an opening made in the cortex of the lower border of the left kidney, a piece of the kidney cortex of corresponding size having been previously removed. The graft was secured by silk ligatures. Eighty-six days later the remaining right suprarenal was removed. The rabbit made an uninterrupted recovery and was apparently normal in all respects.

Twenty-one days after the removal of the remaining suprarenal the rabbit was killed, in order to examine the condition of the graft. It was found that both original suprarenals had been entirely removed, without leaving any stump that might have carried on the function of the gland. No accessory suprarenals were found. The remains of the graft, upon histological examination, were found in the cortex of the right kidney just under the capsule, the estimated thickness of the surviving graft being about 0.8 mm. The cells of the graft appear to belong to the medullary portion of the suprarenal. The rest of the original graft seems to have been replaced by connective tissue. The vascular supply of the surviving cells is good.

ON THE ABSENCE OF A CANE-SUGAR INVERTING ENZYME IN THE GASTRIC JUICE.

By GRAHAM LUSK.

In a former paper by Ferris and Lusk[1] the cane-sugar inverting power of the gastric juice was attributed to free hydrochloric acid, which was shown adequate to effect the results observed. The experiments of Miura were accepted as indicating the absence of inverting enzymes. Recently Widdicombe[2] has said that gastric juice contains an inverting enzyme. In consequence of this statement, the following experiments were instituted: —

Dog I. — 1. Dog had fasted 48 hours. 1 c.c. of fresh gastric juice (stimulus largely psychical, less than 5 grams of meat fed) was collected in drops from a gastric fistula 40 minutes after the stimulus. Free acid present indicated by tropæolin 00. Mixed with 1 c.c. of a 5 per cent cane-sugar solution, and kept at 37° C. Inversion in one hour indicated by the reduction of Fehling's solution.

2. 5 c.c. of the same gastric juice is permitted to digest fibrin until there is no reaction for free acid, then 1 c.c. of a 5 per cent cane-sugar solution is added, and the mixture put in a thermostat at 37° for 23 hours. The proteid was precipitated by the alcohol method, and after neutralization of the filtrate, and evaporation of the alcohol, no reaction for invert sugar could be obtained.

Dog II. — 3. Fasting dog, anæsthesia by morphine and ether. Pylorus tied. Gastric stimulus through 25 c.c. of a 25 per cent solution of alcohol injected into the duodenum (method of Wallace and Jackson). One hour later cardia ligated, stomach extirpated and put in thermostat 4½ hours. 15 c.c. of gastric contents found, no free acid present, but acid to litmus. Filtered. 3 c.c. of the filtrate, with 1 c.c. of a 5 per cent solution of cane-sugar, after standing in the thermostat for 24 hours, showed no inversion.

4. The gastric mucosa of the same dog, with about 10 c.c. of the above-mentioned gastric contents, were digested for 24 hours with 200 c.c. of a 0.4 per cent hydrochloric acid solution. Reaction shows free acid. Fibrin is added, strong proteolysis takes place, and free acid disappears. 3 c.c. of this filtered digest, and 1 c.c. of a 5 per cent solution of cane-sugar, showed no inversion after standing in a thermostat for 24 hours.

[1] Ferris and Lusk: This journal, 1898, i, p. 277.

[2] Widdicombe: Journal of physiology, 1902, xxviii, p. 175.

Pig. — 5. A pig's gastric mucosa was treated as above with 1000 c.c. of 0.4 per cent hydrochloric acid. After 24 hours, no reaction for free acid. 15 c.c. of the filtered digest, to which was added 0.5 gram of cane-sugar, showed no inversion after 24 hours in the thermostat.

The above experiments demonstrate that no cane-sugar inverting enzyme exists in the gastric secretion, and confirm the belief that such inversion of cane-sugar as takes place in the stomach is alone due to the presence of free hydrochloric acid.

IMPROVED CAGE AND DIET FOR USE IN METABOLISM EXPERIMENTS ON DOGS.

By WILLIAM J. GIES.

Cage. — After using cages of several types of construction during the past few years, the author has improved a simple form by adding various mechanical devices which ensure quantitative accuracy as well as comparative convenience in the collection of excreta (urine, fæces, hair).

Diet. — The improved diet consists of hashed meat, cracker meal, lard and water, with a quantity of *bone ash* equivalent to that from a moderate amount of bone. Bone ash is a very desirable addition to the diet of dogs in metabolism experiments, and has already been used in the experiments reported by Taltavall and Gies at the previous meeting of the society, and in the experiments reported by Hawk and Gies at this meeting.

The addition of bone ash to the diet of dogs increases the bulk of the fecal matter and makes its discharge more frequent and regular. The fæces have the typical consistency and appearance of the fecal matter eliminated from dogs subsisting on a diet containing bone. Bone ash does not introduce into the diet anything that is injurious to the dog, either mechanically or chemically. The fondness of dogs for bones is well known. There is never any diarrhœa as a result of the presence of the bone ash in the food. The fæces are eliminated in lumps that do not adhere to the cage, but are very easily and completely removable from it. They dry very readily on the water bath *in a few minutes* and may be quickly and easily ground to a

fine, fluffy powder. Charcoal marks off period-fæces very distinctly when bone ash is fed, because of the sharp color contrasts. Ten grams of bone ash to a medium-sized dog in the average daily diet suffices to produce the effects desired.

That the bone ash has little effect on the taste of the food or on the dog itself, is evident from the fact that I have succeeded in feeding large amounts of the material to several animals: one dog weighing 17 kilos took daily as much as 100 grams of bone ash admixed with 250 grams of hashed meat, 70 grams of cracker meal, 30 grams of lard, and 500 c.c. water, without showing any observable effects whatever, except more frequent and abundant defecation. Although such a mixture, containing an excess of the bone ash, looks much like powdered chalk and water, the dog ate it as readily as it did the hash without it. The animal referred to was a well nourished animal to begin with. Moderate amounts of bone ash do not appear to interfere with digestion or absorption. I have not yet determined the effect on excretion of earthy salts in the urine. Nearly all of these substances in the bone ash seem to appear in the fæces, however.

DEMONSTRATION OF WORKING MODELS OF THE CIRCULATION.

By YANDELL HENDERSON.

In the first model, valves of sheet rubber anatomically similar to the mitral and semilunar (together with a reservoir supplying water, a rubber bulb serving as pump, and an air-chamber representing arterial elasticity) are so arranged that the movements of the valves can be observed under varied conditions.

The second model is a mechanical device consisting of a pump, air-chamber, stop-cocks, by-passes, etc., designed to determine the relative systolic volume, arterial elasticity, peripheral resistance, and the factors incident to conditions of stenosis and regurgitation, when the model is arranged to give pressure curves similar to intraventricular and pulse tracings.

DEMONSTRATION OF RABBIT'S NERVES, SHOWING THE EFFECT OF LIGATION UPON VITAL STAINING.

By S. J. MELTZER.

A single ligation of a nerve has no influence upon the staining of the nerve on either side of the ligature. When, however, two ligatures are applied, the section of the nerve between the ligatures remains free of color, while both ends are stained. This is the case, even if the section between the ligatures comprises nearly the entire length of the nerve.

ON THE ENZYME OF THE THYMUS.

By WALTER JONES.

A kilogram of finely divided thymus gland was suspended in twice its weight of chloroform water and allowed to remain for five days, at the body temperature. The product was then treated with a few drops of acetic acid, heated to boiling for the coagulation of the proteids, and the filtered solution made strongly alkaline with ammonia, when the usual precipitate of magnesium ammonium phosphate occurred. This was filtered off, and the solution treated with silver nitrate in ammonia. The profuse gelatinous silver precipitate thus produced was submitted to the scheme proposed by Krüger and Solomon for the separation of xanthine bases, and there were finally obtained $2\frac{1}{2}$ grams of xanthine nitrate and 110 milligrams of hypoxanthine nitrate. As the hydrolytic products of thymus nucleic acid which belong to this group are guanine and adenine, the following experiment was undertaken to show that the xanthine in question results from a decomposition of the nucleoproteid and not from some hitherto unrecognized constituent of the gland. A kilogram of prepared gland was extracted with water and the nucleoproteid precipitated with acetic acid. This was purified by alternate solution in sodium carbonate and precipitation with acetic acid until a neutral opalescent solution was finally obtained, free from all constituents of the gland except the nucleoproteid, and the enzyme which adheres to it. This material was treated with chloroform and allowed to digest at the body temperature. At the end of sixteen hours the presence of xanthine bases could be shown. After a week, the pro-

ducts of the digestion were examined by the method stated above. 1.7 grams of xanthine nitrate and 70 milligrams of hypoxanthine nitrate were finally obtained. The production of phosphoric acid was also shown.

ON THE ENZYME OF THE SUPRARENAL GLAND.

BY WALTER JONES.

THE prepared gland was treated with four times its weight of chloroform water, and maintained at the body temperature for a week. The product was then treated with acetic acid, heated to coagulate the proteids, and the filtered solution evaporated to a small volume under diminished pressure. The deposited sediment was found to consist principally of xanthine, but contained also a small quantity of hypoxanthine. The absence of other xanthine bases was definitely shown. The nucleoproteid was then prepared by extracting the gland with water and precipitating with acetic acid. By alternate solution in sodium carbonate and precipitation with acetic acid the material was freed from soluble constituents of the gland, and finally placed in the thermostat in neutral solution with chloroform. At the end of a week the product was examined by the method described in connection with the thymus gland, and with the same results.

It is thus shown that there is present in the thymus, and also in the suprarenal gland, an enzyme which exerts its activity on the nucleoproteids of the glands giving rise to phosphoric acid and xanthine bases, and that the xanthine bases formed under the influence of the enzyme are not the same as those formed when the nucleoproteids are boiled with dilute acid.

THE PHYSICAL FACTORS CONCERNED IN URINE FORMATION.

BY TORALD SOLLMANN (WITH THE COLLABORATION OF R. A. HATCHER).

I. *The excretion of chlorides by excised and perfused kidneys:*

1. If saline solutions are perfused, the chloride-content of the perfusing fluid and of the ureter filtrate are identical.

2. If mixtures of defibrinated blood and sodium sulphate are cir-

culated, the ureter filtrate contains almost as much chlorides as the serum. The slight retention which occurs is different in kind as well as in degree from the retention which occurs in the living animal. This "living retention" has been lost.

3. This is not due to changes in the chlorides of the serum produced in defibrination; for defibrination has no effect on the chloride retention of living animals. It must be due to injury to the kidney produced by very brief interruption of the renal circulation.

II. *Changes occurring in the kidney during perfusion with one per cent sodium chloride under uniform conditions.*

These are shown to be mechanical, due to kinking of the renal vessels. A period of two hours exists when the vein and ureter flow are almost precisely constant.

III. *The influence of the injection pressure.*

The volume of the kidney, and the vein and ureter flow, are parallel to the arterial pressure.

IV. *The effect of compressing the renal vein.*

Occlusion of the vein practically abolishes the ureter flow. This begins quite suddenly when the vein-pressure reaches 50 cm. of water. The vein flow stops suddenly when the vein-pressure reaches 70 cm. of water. A kinking of the ureter and of the vessels must occur at these pressures.

V. *Results of occluding the ureter.*

A sudden relative decrease of ureter flow occurs when the ureter-pressure reaches 60 cm. of water. The urinary tubules must be partly occluded by kinking at this pressure. The vein flow shows a comparatively small diminution, and the oncometer a small increase. The maximal ureter-pressure is always less than the injection pressure.

VI. *When the injection is made through the vein*, but little solution can be forced through the vessels.

VII. The vein and ureter flow are diminished by *increase of viscidity* of the solution.

VIII. *Concentration of the injection fluid.*

The flow from vein and ureter are parallel to the concentration of the injection fluid. The effect is very great. The oncometer shows definite oscillation; the final volume of the kidney is not greatly altered.

IX. *Isotonic solutions, compared with one per cent sodium chloride.*

The following produce definite changes, which are not, however, very great: cane sugar, glucose, potassium, ammonium.

The following cause a very considerable diminution in vein and ureter flow: urea, calcium, barium, acid, alkalies, carbonate. Magnesium causes an increase. Urea also causes a decrease of kidney volume. After flushing with salt solution, the vein flow recovers with most of the solutions, but not the ureter flow.

X. *Various substances added to two per cent sodium chloride.*

Sodium fluoride and mercuric chloride (1 : 1000) diminish both ureter and vein flow very greatly. No recovery occurs when pure saline is circulated. Sodium arseniate and formaldehyde (1 : 1000) produce absolutely no effect. A number of other substances which were tried, had little, if any, action.

EXPERIMENTS ON THE PRECURSORS OF URINARY INDICAN.

FRANK P. UNDERHILL.

The recent experimental observations of Ellinger and his coworkers have made it probable that the indican of the urine does not arise from nitrogenous products of tissue decomposition (as Blumenthal and others have assumed), but rather owes its origin to the activity of bacteria which form indol in the intestine. They have also demonstrated that tryptophan may be a precursor of indol in putrefaction. The discovery of the constitution of tryptophan as skatol-amido-acetic acid by Hopkins and Cole, and their announcement that the Adamkiewicz (glyoxylic acid) reaction of proteids is attributable to the tryptophan group in the latter, has afforded the occasion for the present experiments. Various proteid substances yield the Adamkiewicz reaction with different degrees of intensity, — in some cases with entirely negative outcome. Of the substances which fail to give the test, gelatin is the most familiar. Feeding experiments on dogs have indicated, in conformity with theoretical considerations, a marked decrease in the excretion of indican when gelatin is the chief nitrogenous constituent of the diet. The relationship between the *quality* (as well as the quantity) of the nitrogenous foods ingested and the indican output in the urine has been demonstrated; and thus a new factor has been introduced into the consideration of the physiological significance of indican formation. The details of the investigation will soon be published.

THE INFLUENCE OF HEMORRHAGE ON PROTEID CATABOLISM.

By P. B. HAWK and WILLIAM J. GIES.

The authors have recently completed some experiments in this connection on dogs brought to a condition of nitrogenous equilibrium. Blood was withdrawn from the femoral artery, or one of its branches, while the animals were under ether-chloroform anæsthesia. The influence of anæsthesia and of our usual operation, with or without ligaturing the artery, was determined on each dog in order to check the results of blood-letting under anæsthesia. The operations were conducted under aseptic conditions. The diet was always eagerly taken and was the same as that referred to in another report at this meeting by Dr. Gies. The periods of recuperation were from one to two weeks in length. In one experiment the animal was under observation continuously for eighty-five days, and was subjected to five losses of blood equal each time to from 2.5 per cent to 3.5 per cent of the body weight.

In spite of losses of blood-nitrogen varying between 8 and 15 grams, hemorrhages of about 3 per cent of the body weight caused, among other effects, (1) diminished secretion and decreased specific gravity of the urine at first, the reverse after twenty-four to forty-eight hours; (2) a slight temporary increase in the amount of nitrogen and sulphur in the urine, and a decrease in the quantity of excreted phosphorus.

The amount and consistency of the fæces were unaffected. The changes in the amounts of excreted nitrogen, sulphur, and phosphorus were registered solely in the urine.

In the experiments in which the effect of anæsthesia with operation was determined *before* hemorrhage, the catabolic effects above referred to were almost as great. Anæsthesia with operation *after* hemorrhage caused a reverse effect — decrease instead of increase.

Repeated hemorrhages from the same animal resulted in (1) cumulative quantitative catabolic effects in harmony with those after single losses of about 3 per cent of the body weight, and were followed by (2) steady decline in body weight, and (3) gradual increase in daily volume of urine, even when the animal ate the same amount and kind of food as at the beginning of the experiment. (4) Nitrogenous equilibrium seemed to be repeatedly established in a few days

after each hemorrhage, on successively lower planes, by the original amount of food.

After successive hemorrhages at intervals of a few days the content of nitrogen and sulphur in the blood, as well as specific gravity and number of erythrocytes, gradually diminished, whereas the leucocytes steadily increased in number. The phosphorus content remained about the same.

Experiments on the flow of urine directly from the kidney confirmed previous statements to the effect that a hemorrhage of 3 per cent of the body weight entirely stops urinary formation for about a half-hour.

Our data confirm the general metabolic results obtained years ago by Bauer and others, and disagree with the contrary conclusions lately announced by Ascoli and Draghi.

NITROGENOUS METABOLISM AFTER SPLENECTOMY.

By LAFAYETTE B. MENDEL (with R. B. GIBSON).

An opportunity to carry out a series of experiments on metabolism in man after splenectomy has enabled the writers to control the observations on splenectomized animals reported to the Society in 1899 by Mendel and Jackson. It was demonstrated by them, contrary to current opinions expressed in physiological literature, that the spleen is by no means the chief organ involved in uric acid production in the living body, if, indeed, it normally plays any part whatever in this process. In the present case, a study extending over many days was made of the composition of the urine under known conditions of diet. The chief points of interest indicated by the detailed protocols presented are: the normal character of the curves of postprandial hourly excretion of uric acid and other nitrogenous constituents of the urine; the retention of chlorides during febrile conditions; the undiminished capacity of the organism to form uric acid from its precursors (purin bodies of the food); the relatively large output of uric acid of "endogenous" origin on a purin-free diet; the pronounced elimination of urobilin at times. The writers are inclined to attribute some of these features to impaired hepatic functions, of which the clinical data (ascites, cir-

rhosis, urobilinuria) give evidence. Should further studies likewise indicate a high "endogenous" uric acid output in hepatic disease, the quantitative determination of the uric acid of the urine after a strict purin-free diet may prove of value in clinical diagnosis. A complete record of the investigation will be published.

COEFFICIENTS OF DIGESTIBILITY AND AVAILABILITY OF THE NUTRIENTS OF FOOD.

By W. O. ATWATER.

FOUR years ago, an attempt was made to find coefficients which would express the proportions of the nutrients of common food materials which are digested and made available to the body when eaten in ordinary mixed diet by people in good health.[1] The data were found in the results of ninety-three digestion experiments with men, in which the amounts of protein, fats, and carbohydrates in the food were compared with the corresponding materials in the intestinal excreta. The latter include both the undigested residues of the food and the residues of metabolic products. The coefficients of digestibility, properly speaking, would be found by subtracting the undigested residues from the food. The coefficients of availability are found by subtracting the total excreta from the total food. In accordance with common usage, these coefficients of availability are also designated as coefficients of digestibility. In the article referred to, coefficients were given for different classes of food materials, as meat, fish, and dairy products, making together one class, cereals a second, dried legumes a third, sugars and starches a fourth, and so on. From the data thus observed, factors were assumed for the total food of ordinary mixed diet. Lately, the results of four hundred and eleven such experiments, made during the past nine years, have been collated and the coefficients of availability calculated.[2] The factors actually found in the average of all these experiments agree very

[1] ATWATER and BRYANT: Report of the Storrs (Conn.) Experiment Station, 1899, p. 73.

[2] These experiments were made in a number of different laboratories, and belong to an inquiry regarding the nutrition of man, which is being carried out in different parts of the United States under authority of the U. S. Department of Agriculture.

closely with those assumed from the smaller number, as appears from the following comparison: —

COEFFICIENTS OF AVAILABILITY OF NUTRIENTS.

	Protein.	Fat.	Carbo-hydrates.
	per cent	per cent	per cent
Proposed factors	92.0	95.0	97.0
Factors found in average of 411 experiments	91.1	94.8	96.8

The kinds of food used in these experiments were such that the proportions of vegetable food materials were, on the whole, larger than in what might be called "ordinary mixed diet" in the United States. The coefficients for protein are smaller in the vegetable than in the animal foods. The difference between the proposed factor for protein, 92 per cent, and that found in these experiments, 91.1 per cent, may be easily accounted for by this difference in proportions of food materials.

The inference is that the proposed coefficients represent very nearly the actual average availability or digestibility of the nutrients of ordinary mixed diet.

THE INFLUENCE OF HEMORRHAGE ON THE FORMATION AND COMPOSITION OF LYMPH.

BY E. R. POSNER AND WILLIAM J. GIES.

AFTER hemorrhage, as is now well known, fluid passes from the tissue spaces into the blood-vessels, and the volume of the blood is soon restored. At first the percentage content of water in the regenerating blood is higher than usual, at the same time its percentage content of organic solids is subnormal. We have attempted to ascertain the facts of post-hemorrhagic lymph flow and composition in the region drained by the thoracic duct, having been led to this research by the contradictory results obtained a few years since by Tscherewkow and Hoche.

We have had four classes of experiments thus far, with controls: the influence of hemorrhage (1) on the production of lymph in

(*a*) recently fed and in (*b*) fasting animals, and (2) on the effects induced by lymphagogues, like (*c*) sodium chloride and (*d*) leech extract.

Thus far after hemorrhage we have always observed diminished flow of lymph from the thoracic duct, in recently fed as well as fasting dogs. After a 2.5 per cent hemorrhage, the injection of 22 grams of sodium chloride (dissolved in 75 c.c. H_2O) into a dog weighing 28 kilos gave lymphagogic effects as decided within a few minutes, as in a normal animal. The same effect was noted after a hemorrhage of 3 per cent of the body weight, followed by the injection of leech extract (27 c.c. of 0.8 per cent NaCl extract of eleven leech heads into a dog weighing 19 kilos). In the latter cases the increased production of lymph was noted after the hemorrhage had previously much diminished its flow.

We have not yet observed any regularity in the proportion of organic solids eliminated in the lymph under these conditions, except after injection of lymphagogues. Without lymphagogues, hemorrhage was followed in some instances by decrease of organic solids, in others by increase, but in each of these cases the inorganic matter was the same in amount throughout, and equal to the proportion of ash in the blood.

The hemorrhages have amounted usually to about 3 per cent of the weight of the animal. In some cases the effects of smaller and repeated losses of blood have been ascertained.

Additional experiments are in progress, in which a study is being made of the content of proteid, fat, and other constituents of the lymph collected under conditions similar to those indicated above.

REPORT OF AN EXPEDITION TO CRIPPLE CREEK AND PIKE'S PEAK TO STUDY THE EFFECT OF ALTITUDE ON THE BLOOD.

By GEORGE T. KEMP.

The party was composed of Professor Kemp, Messrs. O. O. Stanley and E. R. Hayhurst, Assistants in Physiology, C. E. Harris, Fellow, Miss Henrietta Calhoun and Mr. E. L. Draper, graduate students. Each had had at least a year's experience in making the observations assigned them, each was in good health, and each was the subject of

a daily experiment, involving the following points: count of the red corpuscles, count of the leucocytes, determination of the numerical ratio of the blood-plates to the red corpuscles, determination of the hæmoglobin, determination of the specific gravity, special microscopical examination with one-twelfth oil immersion lenses of any interesting structure which presented itself.

The party first took records at Champaign (700 ft.), then went direct to Cripple Creek (9,400 ft.), thence to Pike's Peak (14,200 ft.), thence returned to Champaign.

The results given are from a composite curve made by averaging the daily records on the blood of each of the six in the party. Where fluctuations occur in the curve they represent fluctuations in the curves of the majority of the party, not excessive variations in the curves of one or two of the individuals.

Red corpuscles. — Upon going from Champaign to Cripple Creek there was no change in the number of red corpuscles on the afternoon of the day of arrival.

In two days the count rose from 5,100,000 to 5,900,000, and maintained this level for three weeks, fluctuating between 6,100,000 and 5,600,000.

At Pike's Peak there was a rise of 400,000 the first day, then a slight fall, and the level was maintained at about 6,000,000 for eight days, — the duration of our stay.

From Pike's Peak we returned to Champaign, and six days after leaving the Peak the count had fallen to 5,400,000, and was still falling.

It is a known fact that morning counts of red corpuscles run slightly higher than afternoon counts. On Pike's Peak these differences were regularly enormous, amounting in individual cases to 1,000,000. It is important to remember this in counting the blood-corpuscles of patients treated at high altitudes.

From the end of the first week at Cripple Creek microcytes were present in sufficient number to attract attention; later they became numerous.

Nucleated red corpuscles were observed in isolated instances; they were only exceptionally present, and always too few to count.

The changes in the blood-plates are more marked than those in the red corpuscles. This is true both qualitatively and quantitatively.

The change in the ratio of the blood-plates to the red corpuscles is more striking than any other change in the blood; it is greater in

degree, more regular in progression, and of longer duration than the change in the number of the red corpuscles.

This was observed, without exception, in the blood of six healthy individuals, so it practically represents a law.

It is fair to assume that this may be taken as an index to the changes normally produced in the blood by altitude, and may be used to determine whether the blood of anæmics treated at high altitudes is following normal lines of improvement.

At high altitudes the fluctuations in the count of the red corpuscles at different times of the day is enormous. The ratio of the blood-plates to the red corpuscles is not affected by these fluctuations in any marked degree.

The ratio of the blood-plates to the red corpuscles may be determined more easily and with smaller error than the count of the red corpuscles by the hæmocytometer. This ratio cannot be determined in dry preparations, owing to the adhesion of the plates.

The first change indicating a connection between the blood-plates and the red corpuscles was the presence of a considerable number of large plates, some of them closely approximating the size of red corpuscles. They were mostly oval in form, delicate (thin) in structure, and colorless. At about the same time the number of small red corpuscles became so large as to attract attention. Later (about ten days after reaching Cripple Creek), colored blood-plates began to appear, and were more or less in evidence during our stay at high altitudes, but were most numerous during the second and third week. In the same drop of blood could be seen all intermediate stages between the plates and the reds.

The blood-plates were tested from time to time by Deetjen's method, with sodium hexaphosphate on agar. They were seen to throw out the processes which Deetjen called amœboid. This was also true of the delicate plates containing hæmoglobin, but was not true of the thicker red corpuscles, either young or old.

Altitude appeared to have no effect on the leucocytes, either as to number or kind. This would indicate that the plates are not related to the leucocytes.

The amount of hæmoglobin in the blood is a very unsafe index for the number of red corpuscles.

In the long run, and in a general way, the hæmoglobin may be said to go with the red corpuscles, but the daily variations were often in the opposite direction. At Cripple Creek the rise in the amount of

hæmoglobin was fully twenty-four hours in advance of the increase in the number of the red corpuscles.

The daily fluctuations in the hæmoglobin were much less than the daily fluctuations in the red corpuscles, showing that when fewer red corpuscles were circulating each carried an increased per cent of hæmoglobin. This point, taken in connection with the morning and afternoon fluctuations in the number of red corpuscles, suggests a new function for the spleen. It seems incredible that 1,000,000 corpuscles per cubic millimetre of blood should be built up and broken down daily. It is far more likely that they are temporarily withdrawn from the circulation. It is an old observation that many faintly colored red corpuscles are often found in the spleen. Our observations show over and over again that the amount of hæmoglobin per corpuscle varies in the same blood from day to day; hence we infer that it is lost by one corpuscle and taken up by another, and we suggest that this transfer may take place in the spleen.

Parallel observations on the specific gravity, hæmoglobin, and red corpuscles show that the increase, per cubic millimetre, of the latter cannot be accounted for by different degrees of concentration of the blood.

It is a disputed point as to whether the specific gravity can be taken as an index to the number of red corpuscles. Our observations show repeatedly that it cannot, especially where such variations occur as are observed at high altitudes.

EFFECT OF INTRAVENOUS INFUSION OF SODIUM BICARBONATE AFTER SEVERE HEMORRHAGE.

By PERCY M. DAWSON.

The fluids infused contained sodium chloride and sodium bicarbonate in varying amounts (0.5 to 0.8 per cent and 0.1 to 1.0 per cent respectively). During anæsthesia with morphia and ether, the animals (dogs) were bled through the carotid artery and then infused through the jugular vein.

In recording the systolic and diastolic pressures, the exclusive use of the valved or of the Hürthle manometer was found to be unsatisfactory. The former, though accurate, is slow, and cannot be used for obtaining pulse-curves, while the latter (under the conditions

present in these experiments) is often quite unreliable. The author therefore connected the "Hürthle" with one femoral artery and the valved manometer with the other, and both instruments with the same pressure bottle, so that at any time the readings of the two might be compared.

From the data obtained, it is seen that solutions containing sodium bicarbonate bring about an increase in cardiac action, thereby restoring the blood-pressures (systolic and diastolic) to a higher level than when sodium chloride alone is employed. When a solution containing 0.8 per cent NaCl and 0.25 per cent $NaHCO_3$ is infused slowly, the beneficial action persists for a considerable time.

RICIN.

By T. B. OSBORNE and L. B. MENDEL.

The proteins of the castor bean are an albumin, a globulin, and a proteose. The two latter, when completely separated from the albumin, are without toxic action. These two bodies constitute much the greater part of the protein matter of this seed, although the amount of albumin is not inconsiderable. The globulin was separated from the saline extracts of the seed by dialysis, the albumin by saturation with magnesium sulphate (some albumins of vegetable origin are precipitated by saturating their solutions with magnesium sulphate), and the proteose by precipitation with alcohol, after freeing the solution from salts by dialysis.

The toxicity of the purified albumin was very great, 0.002 milligram per kilo being sufficient to cause the death of rabbits when subcutaneously injected, or $\frac{1}{20}$ the minimal quantity required in the intravenous introduction of Cushny's preparation. The post-mortem appearance of the tissues was characteristic in every case. The ricin preparation also possessed very marked agglutinating and sedimenting properties towards mammalian red corpuscles.

Our preparation of ricin was completely soluble in distilled water, coagulated slowly between 70° and 80°, gave all the characteristic reactions of proteins in a very pronounced manner, contained C 49.02 per cent, H 6.80, N 14.54, and throughout behaved exactly like a protein body.

These results in the main confirm those obtained by Cushny; and considered in connection with the minimal toxic dose, they lend little support to the recently advanced view that the toxic substance is a non-proteid body. It is our immediate intention to prepare a much larger quantity of this most powerful poison, if possible in a purer condition, in order to obtain more positive evidence respecting its true nature.

THE EFFECTS OF A SUBCUTANEOUS INJECTION OF ADRENALIN ON THE EYES OF CATS WHOSE SYMPATHETIC NERVE IS CUT, OR WHOSE SUPERIOR CERVICAL GANGLION IS REMOVED.

By S. J. MELTZER.

When the sympathetic is cut, a subcutaneous injection of adrenalin causes a retraction of the nictitant membrane, and no change is seen in the size of the pupil or the width of the palpebral fissure. When, however, the superior cervical ganglion is removed, an injection causes a strong dilatation of the pupil, a considerable widening of the palpebral fissure, and a retraction of the nictitant membrane.

ON THE INFLUENCE OF ETHER ANÆSTHESIA.

By P. B. HAWK.

Three phases of the subject have been studied as follows: (1) Glycosuria following ether anæsthesia; (2) Changes in the blood produced by ether anæsthesia; (3) Influence of ether anæsthesia upon urine flow, and the excretion of nitrogen and chlorine.

The experiments were all made upon dogs, the length of the experiments varying from twelve to thirty-nine days. On normal animals the plan was to bring the organism to nitrogen equilibrium by means of a suitable diet, then induce ether anæsthesia for periods of various lengths. The periods thus far investigated have ranged in length from thirty minutes to four and a half hours.

A slight diuresis invariably followed, and accompanying this diuresis was a small increased nitrogen output and a large increase in

the chlorine excretion. Glycosuria always occurred after ether anæsthesia.

In general, the changes noted in the blood after anæsthesia were an increase in the number of red corpuscles, and a lesser increase in the number of leucocytes; the hæmoglobin was more irregular. In the course of a few hours, the red corpuscles became normal in number, whereas the leucocytes increased rapidly and produced an extensive leucocytosis in from three to five hours.

Cannulas inserted in the ureters of normal dogs showed a very slow urine flow during anæsthesia periods of from six and one-half hours to eleven and one-half hours. In one case the flow entirely ceased in three and one-half hours. The urines voided in these experiments showed a very high content of nitrogen and sugar. The red corpuscles were increased from 30 per cent to 50 per cent in these experiments, and the leucocytes were greatly decreased in number. The body temperature of a dog at the end of an anæsthesia period of eleven and one-half hours was 23° C.

Experiments have been made on animals subjected to various periods of fasting. In the case of a dog etherized, on the twenty-second day of fasting a strong diuresis was noted, but, contrary to the conditions obtaining in normal animals, this diuresis was unaccompanied by any signs of glycosuria. The changes produced by ether anæsthesia in the blood of dogs during periods of fasting of from one to twenty-two days, are similar to the changes produced in the blood of normal animals, *i.e.*, an increase in red corpuscles and the accompanying leucocytosis.

THE END-PRODUCTS OF SELF-DIGESTION OF ANIMAL GLANDS.

By P. A. LEVENE.

The results of the analysis of the crystalline end-products of self-digestion of the pancreas gland and of the liver are presented in this communication. Thus far only the amino acids were analyzed. The pancreas was subject to self-digestion in a 0.5 per cent solution of sodium carbonate, the liver in 0.2 per cent solution of acetic acid. Alanin, amino-valerianic acid, leucin, glutamic and aspartic acids, tyrosin, and phenylalanin could be identified. The presence of α-pyrolidin-carbonic acid could not be established with certainty.

THE END-PRODUCTS OF TRYPTIC DIGESTION OF GELATINE.

By P. A. LEVENE.

THE object of the investigation was to compare the composition of peptone with that of native proteid and with that of proteoses. It was found that the molecule of gelatose contained more glycocal than that of gelatine. Gelatine peptone contained less glycocal than gelatose. In order to explain these observations, the crystalline products of digestion were studied.

There were found glycocal (in very large quantities), leucin, glutamic acid (in smaller quantities), phenylalanin and a substance of the composition of inactive pyralidin-carbonic acid :

	Calculated for $(C_{10}H_{16}N_2O_4)CuH_2O$	Found
H_2O	10.99	11.11

For the dry substance:

C	41.16	40.66
H	5.49	5.70
N	9.62	10.00
Cu	21.66	21.70

The copper salt differed in appearance from that of the *a*-pyralidin-carbonic acid.

AN IMPROVED KYMOGRAPH.[1]

By W. T. PORTER.

THE improved kymograph (Fig. 1) consists of a drum revolved by clockwork and also arranged to be more rapidly revolved or "spun" by hand.

The drum is of aluminium, cast in one piece, turned true in the lathe to a circumference of 50 cm. The height is 15.5 cm. The weight is about 600 grams. In each head of the drum is placed a thin steel plate pierced to admit a steel shaft about which the drum revolves and on which it may be held at any desired height by a spring clip. The steel shaft passes through both the heavy plates

[1] I am indebted to Mr. C. E. Roy, foreman of the machine-shop, for valuable assistance in the improvement of the kymograph.

containing the clockwork and is securely bolted to the bottom plate. The motion of the clockwork is communicated to the drum by a brass sleeve surrounding the lower part of the steel shaft and fastened to the intermediate or sleeve-gearwheel shown in Fig. 1. The sleeve is crowned with a disk upon which rests a brass block, carrying a steel rod which passes through an opening in each head of the drum, and finally is bent over until its blunt steel point rests in a shallow cup on the head of the steel shaft. The lower end of the steel rod is held fast in the brass block by a horizontal screw. The brass block is held fast to the disk of the revolving sleeve by a vertical screw, the head of which presses against the under surface of the disk. When these screws are set, the revolving sleeve, the brass block, the steel rod, and the drum form one rigid piece, the entire weight of which is suspended on the point of the steel rod where it bears on the top of the steel shaft. Friction on the steel shaft occurs only at the point of the steel rod, at the thin steel plate in each head of the drum, and at the bearing surfaces of the sleeve, which are made as small as possible.

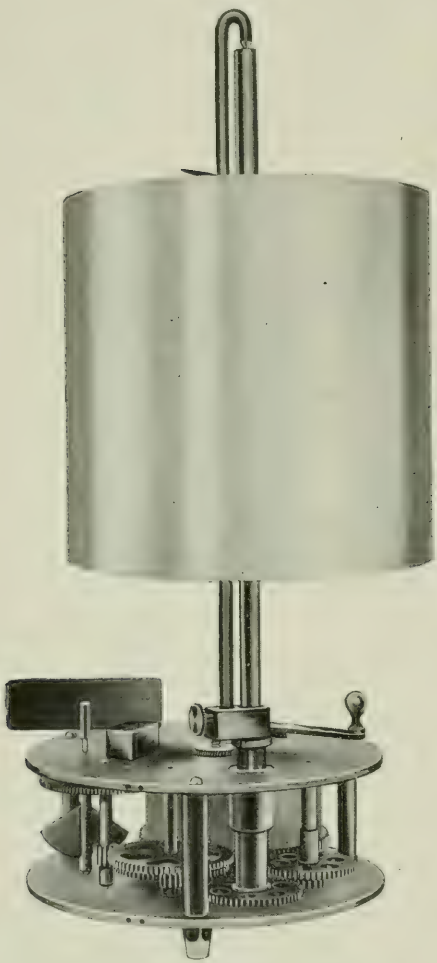

FIGURE 1.

When the vertical screw is given several full turns from right to left so that its head no longer touches the disk of the revolving sleeve, and the side screw is given a half turn from right to left, the brass block may be raised until it no longer rests on the sleeve-disk;

it may be held in its new position by a half turn of the side screw from left to right. The brass block, steel rod, and drum will now be free of the sleeve, and will rest suspended on the point of the steel rod. The drum may now be "spun" by hand. A single impulse will cause the drum to revolve more than a minute, making more than one hundred revolutions. The speed in any one revolution, except at the beginning and the end of the series, will be practically uniform.

The clockwork consists of a stout spring about six metres in length, driving the chain of gears shown in Fig. 1. A slow and a fast set of speeds are provided. The slow speed is obtained by lowering the sleeve until its collar is flush with the plate covering the clockwork, lowering the brass block until it rests on the sleeve-disk, fastening the block to the steel rod by a half turn of the horizontal screw, and turning the vertical screw until its head presses against the sleeve-disk. The pinion of the gear shown on the extreme right of Fig. 1 now engages with the gear of the sleeve and transfers to it the motion of the spring-gear. The slow set of speeds gives place to the fast set, when the horizontal screw is released from the steel rod by a half turn of the screw from right to left, the block with the attached sleeve moved upward as far as possible on the steel shaft, and the sleeve and block secured in their new position by a half turn of the horizontal screw from left to right. The pinion of the gear on the right now no longer engages with the sleeve-gear, but runs "idle," and the sleeve-gear engages with the spring-gear directly. To pass from the fast to the slow speeds the horizontal screw is loosened, the block and sleeve dropped as far as possible, and the screw tightened again.

These operations are easily and rapidly performed, though, as in all gear mechanism, an instant's pause is sometimes required to enable the gear teeth to engage. The clockwork should be in motion, without the fan, when the adjustments are being made.

With both fast and slow gearing four fans of different areas may be used. They are slipped upon an extension of the last pinion shaft in the chain. Five slow and five fast speeds (exclusive of spinning) are thus obtained. An additional slow speed (50 cm. per hour) may be obtained with a very large fan. All speeds are regulated by a governor consisting of two heavy metal wings fastened to the same shaft that carries the fan. With one winding, the drum will revolve from about one to about seven hours, or longer, depending on the fan employed.

RESPIRATION SCHEME.[1]

By W. T. PORTER.

The glass cylinder (Fig. 2) represents the thorax. The surface of the water in the glass cylinder represents the diaphragm and movable chest walls; its level may be changed by raising or lowering the large rubber tube, in the free end of which is placed a second glass cylinder, not shown in Fig. 2. The interior of the cylinder above the water represents the thoracic cavity, and the rubber balloon the lungs. The paraffined cork is pierced by a pleural and a tracheal tube. The upper end of the pleural tube enters a rubber tube in the wall of which is a small hole, closed by a short glass rod. Through this hole the pleural cavity may be opened to the atmospheric air. The tracheal tube opens below into the lung, above into a rubber tube, in the wall of which is a small opening, which represents the glottis, and which may be partly or wholly closed by a glass rod. The left manometer shows the intra-thoracic pressure, the right manometer the intra-pulmonary pressure. The normal relations between intra-thoracic and intra-pulmonary respiration may be reproduced with this apparatus. The pressure changes in forced respiration, obstructed air passages, asphyxia, coughing, sneezing, hiccough, and perforation of the pleura may also be studied.

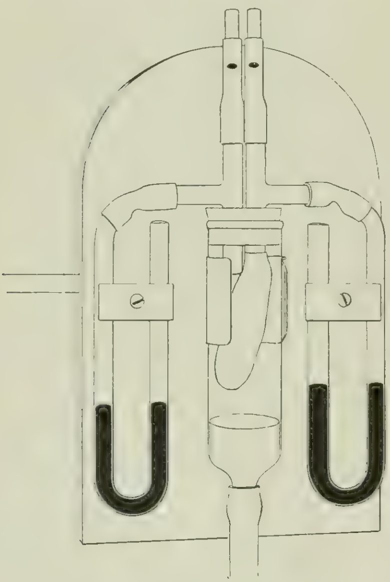

Figure 2.

[1] I am indebted to Mr. Frederick Haven Pratt for assistance in the details of this design.

"MUSCLE WARMER."

By W. T. PORTER.

A disk, supported by a rod, bears three pins (Fig. 3). One of the three pins is prolonged and bent at a right angle near its lower end. To the bend is fastened one end of the muscle under experimentation. About the other end is tied a fine copper wire which passes through a hole in the disk to reach a muscle lever. A second opening in the disk is provided with a short metal tube, in which a thermometer is held by a piece of rubber. The bulb of the thermometer may be placed on a level with the belly of the muscle. When these adjustments are complete, a glass cylinder is brought against the under surface of the disk, where it is held in position by the "spring" of the three pins. A beaker or other vessel containing water is now placed beneath the cylinder and raised until the cylinder is sufficiently immersed. The temperature of the muscle is altered by heating or cooling this water. Direct electrical stimulation of the muscle may be made by connecting one electrode with the metal parts of the apparatus and the other with the copper wire attached to the upper end of the muscle.

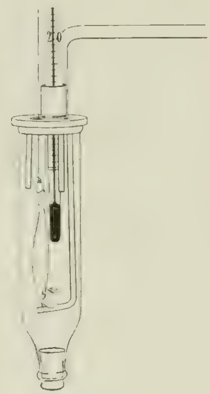

Figure 3.

The Delineation of the Motor Cortex in the Dog. By H. Cushing.

The Simultaneous Action of Pilocarpine and Atropine on the Developing Embryos of the Sea-Urchin and Starfish. By T. Sollmann. This journal, 1904, x, pp. 352–361.

A Method of Demonstrating the Localization of Potassium in Animal and Vegetable Cells. By A. B. Macallum.

A New Head Holder for Rabbits. By Frederic S. Lee.

Demonstration of Expressive Motions in a Decerebrate Animal. By R. S. Woodworth.

Demonstration of the Effects of Subcutaneous Injection or Subconjunctival Instillation of Adrenalin upon the Pupils of Rabbits whose Corresponding Superior Cervical Ganglia are Removed. By S. J. Meltzer.

On the Nucleoproteids of the Brain. By P. A. Levene and L. B. Stookey.

Further Contributions to Muscle Tonus. By T. A. Storey and W. T. Porter.

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NOTES ON THE HEART ACTION OF MOLGULA MANHATTENSIS (VERRILL).

By GEORGE WILLIAM HUNTER, Jr.

INVESTIGATION undertaken by the writer on the nervous system of Molgula manhattensis (Verrill) shows conclusively the existence of a set of connective fibres between the groups of ganglion cells situated on or near the distal ends of the heart tube and the central nervous system.[1] The course of these fibres will be treated at length in a later paper.

The purpose of this paper is to indicate some of the observed phenomena which point toward the physiological connection of the heart and central nervous system in Molgula manhattensis (Verrill).

Historical.

It is not the place of this article to treat exhaustively the subject of cardiac activity. Recent investigation on both invertebrate and vertebrate material seems to demonstrate the myogenic activity of heart-muscle.

The presence of regulative nerves in the vertebrates, both accelerators and depressors, is too well known to need comment. With the exception of one of the lower fishes,[2] these nerves seem to be present throughout the vertebrate group. Accelerator and depressor fibres have also been demonstrated either anatomically or physiologically,

[1] Hunter, G. W., Jr.: Anatomischer Anzeiger, 1902, xxi, p. 241.

[2] Greene, C. W.: This journal, 1902, vi, p. 318.

in several groups of the invertebrates, proofs being especially strong in the Crustacæ and Mollusca.[1]

The actual presence of a regulative apparatus for the heart in the Tunicata has never been proved, although the physiological work of Kruckenburg,[2] Lahille,[3] Lingle,[4] Loeb[5] and others seem to indicate the existence of such an apparatus. These researches, together with that of Schultze,[6] will be treated when under direct reference.

The Normal Heart-beat of Molgula Manhattensis.

It was the endeavor of the writer first to obtain some light on the normal heart-beat of Molgula. Four factors must here be taken into consideration: first, the normal rate of the heart-beat in an advisceral direction[7]; second, the number of heart-beats in an abvisceral direction; third, the number of pulsations in either given direction before reversal; and, fourth, the duration of the rest period just previous to a reversal of the heart.

The normal ab- and advisceral rate of beat. — The following data were obtained by counting the heart-beat in each individual examined, for a period of time at least ten minutes in duration. An average

[1] Dogiel, Jean: Archiv für mikroskopische Anatomie, 1877 xiv, p. 59; Yung, E.: Archives de zoologie expérimentale, 1881, ix, p. 421; Conant and Clark: Journal of experimental medicine, 1896, i, p. 341; Yung, E.: Archives de zoologie expérimentale, 1878, vii; Dogiel: Comptes rendus, 1876, lxxxii, pp. 1117, 1160; Ransom, W. B.: Journal of physiology, 1884, v, p. 261; Foster, M., and Dew-Smith, A. G.: Proceedings of the Royal Society, London, 1875, p. 318; F. Bottazzi and P. Enriques: Archives italiennes de biologie, 1900, xxxiv, p. 111; R. A. Budington: Unpublished work on Mollusca.

[2] Kruckenburg, C. Fr. W.: Vergleichend-physiologische Studien, zu Tunis, Mentone, und Palermo, 3 Abt., Heidelburg, 1880.

[3] Lahille, F.: Contributions à l'étude anatomique et taxonomique des Tuniciers, Dissertation, Paris, 1890.

[4] Lingle: *vidè* Loeb, J.: Comparative Physiology of the Brain and Comparative Psychology, 1900.

[5] Loeb, Jacques: Einteilung in die vergleichende Gehirnphysiologie und vergleichende Psychologie mit besonder Berucksichtigung der wirbellosen Tiere, Leipzig, 1899; Comparative Physiology of the Brain and Comparative Psychology, 1900.

[6] Schultze, L. S.: Zeitschrift für Naturwissenschaften, Jena, 1901, xxviii, p. 221.

[7] The use of the terms "ad- and ab- visceral" is taken from Schultze. By an advisceral contraction, we mean that the wave of contraction in passing over the heart moves in the direction of the viscera; an abvisceral contraction moves in the opposite direction. The terms endostylar and rapheal might be substituted for the above-given terms.

mean pulsation rate was thus obtained for each individual. Three lots of Molgulæ were examined, between twenty and thirty specimens in each lot, at intervals of time separated by two weeks. Laboratory conditions were kept as nearly alike as possible for all animals. The water temperature was between 21° and 22° C. For purposes of comparison, the Molgulæ were divided into three groups marked large, medium, and small, respectively.

Size of specimens. Mm.	ADVISCERAL. Time per 100 seconds.				ABVISCERAL. Time per 100 seconds.			
	Slowest observed pulsation rate.	Fastest observed pulsation rate.	Average normal pulsation rate.	Av. normal rest period.	Slowest observed pulsation rate.	Fastest observed pulsation rate.	Average normal pulsation rate.	Av. normal rest period.
18 × 25 to 22 × 25	59 : 100	98 : 100	75 : 100	secs. 2+	54 : 100	99 : 100	74 : 100	secs. 2+
15 × 18 to 18 × 25	47 : 100	105 : 100	72 : 100	2+	42 : 100	110 : 100	72 : 100	2+
8 × 9 to 14 × 16	52 : 100	82 : 100	67 : 100	2+	50 : 100	90 : 100	68 : 100	2+
	Average for all specimens.		72 : 100 43.2 : 1 m.	2+	Average for all specimens		72 : 100 43 2 : 1 m.	2+

The average mean normal rate of pulsation obtained from these results (together with all other specimens examined) gives the following for all examined Molgulæ: 43.2 per minute in an advisceral direction, and 43.2 per minute in an abvisceral direction, with a rest period of two seconds between the pulsation periods.

An unexpected feature of the above table is that it shows the smaller individuals to have a slower pulsation rate than those of larger size, and of presumably greater age. This is contrary to Schultze's findings regarding the rate of the heart-beat in species of Salpæ where the body size is a noticeable factor. I personally have not investigated this point in the species of Tunicata available at Woods Hole, where my investigations were carried on. For our purpose, such results may be disregarded, and only the general average obtained from some eighty specimens need to be used.

It is seen, however, that very great individual variations are the

rule, and such results as are tabulated above may be of little value in comparison.

The general agreement of the above results with those of Schultze may be found in the following statement (page 233): "The result of my observations was that the frequency of the abvisceral and advisceral pulsations is in general the same." Kruckenburg, however, finds a difference between the frequency of the ab- and advisceral pulse in certain of the Salpæ.

What is the normal number of heart-beats that take place in a given direction before a reversal, or during a single pulsation period? Can a normal average of the number of pulsations be established, and, when once established, is this number constant for any length of time? — In answer to the latter question, it must be said that constant change is the rule. Many factors, the discussion of which would not be in place here, cause a frequent, and, ofttimes, erratic change in the time (duration) of the pulsation periods in either the ab- or advisceral directions. In making observations with the view of obtaining the average length of time occupied in a given pulsation period in either direction, time enough must be allowed in the series of observations so that the above-mentioned changes can be averaged for a general mean. In making the observations noted in the following tables, each animal was watched, and the heart-beats counted, for at least fifteen minutes in succession, more frequently for from half an hour to an hour; the rate and number of heart-beats were entered in tabular form, and an average made from the total number of ab- and advisceral beats, respectively. Frequently the same heart was counted at a later time, and the results then obtained were compared and averaged with the first record. In this way, an estimate could be formed of the average duration of a pulsation period. The following table, based on the results obtained from the comparison of forty-three Molgulæ, is self-explanatory.

Table showing Number of Pulsations between Reversals in the Normal Heart of Molgula manhattensis.

	Over 200.		200 to 150.		150 to 100.		100 to 50.		50 and under.		Total.
	Ab.	Ad.	Ab.	Ad.	Ab.	Ad.	Ab.	Ad.	Ab.	Ad.	
No. of specimens	2	2	3	3	8	9	19	19	11	10	43
Percentage . . .	4.7	4.7	6.9	6.9	18.6	19	44.2	44.2	25.6	25.2	

A second lot of thirty-eight Molgulæ gave the following results:

	Over 200.		200 to 150.		150 to 100.		100 to 50.		50 and under.		Total.
	Ab.	Ad.	Ab.	Ad.	Ab.	Ad.	Ab.	Ad.	Ab.	Ad.	
No. of specimens	1	1	4	4	9	9	13	13	11	11	38
Percentage . . .	2.8	2.8	10.5	10.5	23.7	23.7	34.2	34.2	28.8	28.8	

Observations by Kruckenburg and Schultze along this line point out (as do the tables given above) the great variability in the length of the pulsation period. Examples of the heart beating in one given direction much longer than in the opposite direction are noted by the above-mentioned writers. This lack of balance in the duration of the alternating pulsation periods has been noted for many cases in Molgula.

It is the exception, rather than the rule, to have a pulsation period in an abvisceral direction exactly balance the next succeeding pulsation period in an advisceral direction. It is only when a large number of heart-reversals are taken into consideration that we find the number of beats in the two series approximating each other in duration and in the number of alternating beats.

What is the duration of the rest period between reversals of the heart in Molgula? — The rest period is also a matter of great irregularity, as is shown by the following table:

	Rest period, under 1 sec.		Rest period, 1–2 secs.		Rest period, 3–5 secs.		Rest period, over 5 secs.		Total.
	Abv.	Adv.	Abv.	Adv.	Abv.	Adv.	Abv.	Adv.	
No. of specimens	6	6	18	18	15	12	3	4	40
Percentage . . .	15	15	40	45	37.5	30	85	10	

Forty-two and five-tenths per cent of all observed specimens had a rest period of between one and two seconds duration. The writer believes this condition to represent a normal condition; while the rest period of very long or of very short duration is a pathological indication. A long, and in some cases, at least, a short rest period, is seen in animals that have suffered from the effects of long captivity or from unfavorable laboratory conditions.

The findings of Schultze are in accord with mine regarding the length of the rest period. He, too, finds exceeding irregularity in the length of the rest period.

Is the rhythmical activity of the heart of Molgula myogenic in origin? — The experiments of Lingle, quoted by Loeb,[1] seemed to show that the heart of Molgula, when cut into two pieces, would beat continuously from the uncut ends. If a piece was cut out of the middle of the heart, the ends would continue to beat, but the middle piece would remain passive. These results, obtained over ten years ago, have since been verified several times by the pupils in Loeb's physiology classes at Woods Hole, and by myself in the summer of 1900.

Schultze, however, after a series of careful experiments, came to the conclusion that when the heart of certain of the Salpæ, and that of Ciona intestinalis, was cut into small pieces, these pieces would continue to contract rhythmically.

These findings led the writer to make a more careful series of observations on the heart of Molgula, with a view of determining this point. The heart, with the surrounding tissue, was removed from the body and left in sea-water in covered glass dishes until after the shock effect had worn off. After beating was completely re-established, the two ends of the heart were cut away and the middle piece left in the dish. Frequent observation of these middle pieces revealed no immediate beating in any of the specimens. In a small percentage of cases, however, pulsations of a more or less rhythmical character were observed. Furthermore, if the middle piece of the heart was cut up into smaller pieces, each piece less than 2 mm. square, and proper precautions taken, the smaller bits of heart-tissue were observed to beat. In all the observed cases, the rate of pulsation was much less than the normal rate, or that of the cut ends. When the hearts were cut into small pieces, these pieces were usually from one to two millimetres in width, and extended completely around the heart tube, thus forming small segments of that organ. In the smallest of these pieces the pulsations were usually of a very irregular character, and occurred at infrequent intervals, sometimes from one to two minutes elapsing between pulsations. Beating of the fragments of the above-mentioned material has been noticed five hours after operation; and in one specimen,

[1] Loeb, J.: Comparative Physiology of the Brain and Comparative Psychology, 1900.

a small piece of heart-tissue three millimetres long, taken from the centre of a heart, was observed to pulsate at the rate of thirty-three beats to the minute.

So far as could be seen, when the heart was divided into two nearly equal parts, these pieces uniformly continued to pulsate from the uncut end toward the cut end. In hearts that were so cut as to leave only one-third of the heart intact, beating would still take place from the uncut end. In a very few cases, where a minute fragment was removed from one end, irregular pulsations were observed to take their origin from the cut end of the heart. This latter phenomenon, however, was never observed immediately after the operation upon the heart, and was rare. After the removal of a small strip of tissue from such a specimen as has been mentioned above, the heart would go on beating from the uncut end, and in no observed case did beating again take place from the cut end.

It is the opinion of the writer that with the aid of a Ziegler's [1] compressorium, such as was used by Schultze, it would be possible to diminish the error still more, and to obtain pulsations in a larger percentage of hearts than by means of the methods described above.

The Physiological Evidence for the Connection of the Heart of Molgula with the Central Nervous System.

Does the isolated heart of Molgula beat with a normal rhythm? — It is a well-known fact that the heart of a tunicate, when removed from the body of the animal, and left in normal salt solution, or in salt-water, will continue to pulsate rhythmically. This fact has been noted by all of the earlier workers on the Tunicata, and especially in the Salpæ, as early as 1827 by Chamisso. The recent work of Schultze shows that the isolated heart of Salpa will beat rhythmically for long periods of time, but always with a slower rhythm than the normal rate. Lack of co-ordination is not particularly noticed by Schultze, although he notes changes in the time of duration of the pulsation period.

In the series of experiments performed upon Molgula by the writer, the heart, with the tissues immediately surrounding it, was completely isolated, and left in covered dishes for a few minutes to

[1] Ziegler, H. E.: Zeitschrift für wissenschaftlichen Mikroscopie, 1897, p. 145.

allow for the shock (inhibitive) effect to wear off. The heart so treated very soon establishes a series of rhythmical contractions, the waves of which originate as in the unoperated heart. The rate of these contractions is usually much slower than those of the normal heart, rarely exceeding thirty-two pulsations per minute, against a normal rate of over forty-three per minute. Instead of the normal rest period, and the abrupt change from the ab- to the advisceral pulse and *vice-versa*, as seen in the living normal animal, the change is in this case usually effected by a series of beats from one end of the heart gradually overcoming the established rhythm, and starting an opposition series in the other direction. This method of reversal in the heart of Molgula has been observed by Loeb's pupils at the Marine Biological Laboratory at Woods Hole as the normal method of reversal. It was found by the writer, however, that only very fresh specimens of Molgulæ could be depended upon for results, and as the available supply of animals for this work had to be brought from New Bedford, a point twelve miles from the laboratory, it was often found impractical to use them for careful physiological work. These specimens were used in the laboratory for class work, and it is by no means unlikely that the recorded observations were made on such animals. Furthermore, in the operated hearts, careful observation shows that in a larger percentage of cases both ends of the heart are beating at the same time, although one end may display a markedly stronger rhythm. Irregularity of the rhythm, lack of coordination between the ends, and long pulsation periods only in one direction, the last named phenomenon one of the so-called "death-signs" of Schultze, are all exhibited by the isolated heart.

Does the removal of the ganglion or "brain" of Molgula affect the heart rhythm? — The findings of Schultze are here of great interest. He notes that immediately after the removal of the ganglion from the body a marked depression in the rate of the heart takes place. This depression, however, can also be produced by cutting off a small piece of the tissues surrounding the hyperbranchial groove, or even by removing a small piece of tissue from any part of the animal. In other words, the depression is not dependent upon the particular tissue removed, but upon the amount of tissue removed and the amount of blood lost from the blood-canals. Hence he comes to the conclusion that the heart in the Salpæ is not connected with the central nervous system.

While the loss of blood and "body-fluid" does, undoubtedly, lower

the blood-pressure in the body sinuses and in the heart, and thus lower the rate of the heart-beat, still it is possible to reduce the loss of blood to a minimum by the use of a cauterizing apparatus.[1] A comprehensive series of over fifty experiments with Molgulæ which had been cauterized in the ganglionic region showed the following results. (The effects of cauterization are decidedly more marked when the posterior end of the ganglion and the dorsal nerve chain are destroyed.)

1. In all experiments the immediate result of cauterization is the complete inhibition of the heart in diastole for a shorter or longer period, this period rarely lasting more than a few seconds. After the heart begins to beat again, the rate is usually much slower than the normal. The rhythm, however, increases slowly until it ultimately becomes nearly stationary again, but still at a rate much below the normal. The normal rate of heart-beat for all animals examined (averaged) was 43 + per minute for the ab- and advisceral pulsations. In thirty operated Molgulæ in which the shock effect had worn off, the rate was 31.2 per minute. This rate varied in the individual animals from as low as 18 per minute to as high as 59 per minute.

2. Great irregularity in the heart-rhythm is noticeable. This irregularity may take the form of alternating long with short beats, fluttering beats, almost fibrillar in character, irregular pauses between beats, and pulsation periods of widely differing duration in time.

3. The heart may beat from both directions at once, either with or without a co-ordinating rhythm. Series of rhythmical contractions may be established by both ends of the heart, this state of affairs continuing until one end of the heart seemingly gains control, and asserts itself more strongly, thus causing the contractions to take their origin from that end. Both ends, however, may continue to contract; but one end, giving rise to a series of stronger pulsations, gains the mastery of the blood-current. Often a "return beat" is established by the end of the heart which is beating less strongly, and a rebuff of at least part of the blood contained in the organ takes place before it has left the heart. This latter phase is met with in a large percentage of cauterized animals, or in those in which the

[1] Thanks are due to Prof. C. B. SUMNER, of the College of the City of New York, for the privilege of using a cauterizing apparatus (electrical) devised and used by him in experimental embryological work.

ganglion was carefully removed. In all the above-mentioned cases the origin of the contraction moving in either direction is from the extreme distal ends of the heart tube. In cases where the animal may have lost much blood, and where the heart is not completely filled in diastole, the wave of contraction in a given direction (A) seems to end at a point near the middle of the heart tube, and a second wave of contraction continues the beat to the end of the heart. This second wave arises at the very instant that the next succeeding wave starts from the given end (A). It is possible, however, that this seeming double wave may be only an appearance due to the flaccidity of the heart wall, and consequent wrinkling or folding of the muscle at the middle of the heart.

The following experiment shows some of the above-mentioned points:

EXPERIMENT XXIV.

August 25, 1902. — Ganglion cauterized at 9.15 A. M. Time of observation, 11.15 A. M. to 11.38 A. M.

Abvisceral.		Advisceral.		Abvisceral.		Advisceral.	
Beats.	Time.	Beats.	Time.	Beats.	Time.	Beats.	Time.
	min. sec.		min. sec.		min. sec.		min. sec.
1	0 15	50[1]	4 25	16[2]	1 17	8[1]	0 40
54	4 28	5[1]	0 40	18[2]	1 21	6	0 29
28	1 55	11	0 55	7	0 30	1	?
12	1 0	5	0 25	45	3 5	6	0 35
6[2]	0 30	8	0 33	14	1 2		

[1] Beating irregular in rhythm.
[2] Beating from both ends of the heart at once.

Next observation made at 3 P. M., when the heart made 200 beats between 3.00 and 3.08 P. M., both ends of the heart beating at the same time and with a like rhythm. The stroke in the advisceral direction is stronger. After each advisceral beat a secondary return pulsation in the opposite direction occurs.

3.10 P. M. Pulsations still in both directions at once (25 to the minute), but stronger in the abvisceral direction.

3.34 P. M. Pulsations are stronger in the abvisceral direction, but now show a return stroke after each beat in the opposite direction. A small

bit of débris in the heart was carried by the blood-current, time after time, to the centre of the heart, and was then returned to the advisceral end as if from a vortex, to have the next abvisceral beat repeat the operation. This bit was eventually forced out of the heart on an abvisceral beat. The rate of the heart at this time was 26 to the minnte.

7.52 P. M. Heart beating 16 to 18 per minute in an abvisceral direction.

10.02 P. M. Heart beating at the rate of 7 per minute in an abvisceral direction.

7.55 A. M. Heart pulsations occur at the rate of from 20 to 22 per minute in an advisceral direction with a return beat in abvisceral direction after each pulsation. Specimen lively; reflexes not recovered in region of siphons.

12 Noon. Animal not lively, heart pulsating faintly; very irregular.

2.30 P. M. Animal dead.

Most of the other experiments show all or most of the above-mentioned irregularities, and the above-quoted case is a fair representative of some thirty to forty others.

4. Rhythmical pulsations take place in one direction only, for abnormally long periods of time. As has been previously shown, nearly 43 per cent of normal Molgulæ examined had a pulsation period ranging from fifty to one hundred beats between reversals; over 26 per cent had a pulsation period of less than fifty beats between reversals, and only 3 per cent showed a pulsation period of two hundred beats or over. In the cauterized animals a very different condition exists. The heart, having once established a rhythm in a given direction, will frequently continue to beat in that direction without reversal for as long a period as two to three hours. Several uncounted cases beat for over three hours before reversing; one case showed an actual time of two hours thirty-three minutes, with a total of forty-two hundred pulsations; another specimen gave thirty-three hundred and ninety-five beats in an advisceral direction without a break, the time occupied being one hour and thirty-one minutes. Numerous cases of over five hundred beats were observed. Schultze gives such cases of abnormally long pulsation periods as a sign of approaching death. This does not seem to hold true in Molgula, as in many of the recorded cases the animals showing the above abnormality in the pulsation periods lived for several (two to six) hours after the observations.

5. Especially after the cauterization of the posterior end of the ganglion and the anterior region of the dorsal nerve cord, a series of

double beats, quicker in time than the normal beat and seemingly interfering with it, may be established. The two beats follow each other (in the same direction) so quickly that at first sight it would be said to be a single pulsation instead of two. Close observation, however, shows a second contraction immediately after the first, the two pulsations following down the length of the heart tube only a few millimetres apart.

The effects of electrical stimulation. — The earlier experiments of Dew-Smith and Ransom and the more recent research of Schultze have failed to give any definite information regarding the connection of the ganglion and the heart.

Dew-Smith did get a slight lengthening of the pulsation period in Salpa after stimulation of the ganglionic region; but other writers have tried in vain for results. The difficulties which stand in the way of accurate results are great, and a repetition of some of the experiments of the above-mentioned writers resulted in negative findings. The experiments were, however, far from what they should have been, because of inadequate apparatus, and I hope to be able to repeat them at a future date.

The results obtained with chemical stimuli. — Much previous investigation has been done on the invertebrata by means of chemical stimuli, with especial reference to the connection of the heart and the central nervous system. On the Crustacea, the work of Yung,[1] Dogiel,[2] and others; on the Mollusca, that of Yung,[3] Foster and Dew-Smith,[4] Ransom,[5] and Bottazzi and Enriques;[6] on the Tunicata, that of Kruckenburg,[7] Lahille,[8] Lingle,[9] and Schultze[10] is most note-

[1] YUNG, E.: Archives de zoologie expérimentale, 1878, vii.

[2] DOGIEL: Comptes rendus, 1876, lxxxii, pp. 1117, 1160.

[3] YUNG, E.: *Loc. cit.*, 1881, ix, p. 421.

[4] FOSTER, M., and DEW-SMITH, A. G.: Proceedings of the Royal Society, London, 1875, p. 318.

[5] RANSOM, W. B.: Journal of physiology, 1884, v, p. 261.

[6] BOTTAZZI, F., and P. ENRIQUES: Archives italiennes de biologie, 1900, xxxiv, p. 111.

[7] KRUCKENBURG, C. FR. W.: Vergleichend-physiologische Studien, zu Tunis, Mentone, und Palermo, 3 Abt., Heidelburg, 1880.

[8] LAHILLE, F.: Contributions à l'étude anatomique et taxonomique des Tuniciers, Dissertation, Paris, 1890.

[9] LINGLE: *vide* LOEB, J.: Comparative Physiology of the Brain and Comparative Psychology, 1900.

[10] SCHULTZE, L. S.: Zeitschrift für Naturwissenschaften, Jena, 1901, xxviii, p. 145.

worthy. Such results as bear directly on my observations will be noted when reference to such work is made.

For experimental purposes the following substances were used in solutions varying in intensity from 0.01 to 0.00001. Wherever possible, the solutions were made in normal salt. The substances used were alcohol, atropin, caffein, curari, digatalin, hellebore, muscarin, nicotin, and strychnin. I shall call attention only to such of the obtained results as bear directly on the question of the heart inhibition and acceleration.

For the obtaining of the results which follow, the normal Molgula was placed in a chemically clean glass dish with sea-water, and left undisturbed until the heart-beat became normal. Then the sea-water was carefully drawn off and a solution of the poison in sea-water put in its place, or the poison was added directly to the water, at first contained in the dish, such water having previously been carefully measured. The heart-beat was carefully taken for a few pulsation periods just before the addition of the poison to the water, and again immediately after it had been added. Later observations on the animals were made at short intervals; thus the immediate effects of the drug, as well as the after effects, were noted. For the sake of comparison, wherever it was possible, check experiments were made on animals from which the ganglion had been removed. Inasmuch as such animals sometimes live for days, this comparison was readily possible.

Heart tracings were not obtained because of the lack of apparatus. It is hoped that graphic results may be shown in a future paper.

Owing to the recurrent action of the heart, it is manifestly impossible to plot a curve, showing inhibition or acceleration, that would compare graphically with such a curve as is obtainable in any other of the invertebrates or vertebrates. A graphic representation of heart depression or acceleration can, however, be shown by plotting the numerical result obtained. Such results are seen in the figures following. In all the cases here shown the abscissas give the time marked in periods of one hundred seconds; each one hundred second period having been taken from a single, successive, pulsation period. The ordinates show the number of heart pulsations taking place in a given period of one hundred seconds. The mean, made for the sake of comparison, is obtained by taking the mean of a number of pulsation periods counted just before the poison was added. These diagrams are in no sense to be taken as curves

obtained in the usual manner, but merely as figures which show graphically obtained results and abbreviate explanation.

Experiments with alcohol. — The heart of a normal Molgula is not easily susceptible to the presence of alcohol. Using a solution of 1 part absolute alcohol to 100 parts of sea-water the heart-beat appeared to be neither accelerated nor depressed to any great degree. Depression was noted in some cases. The duration of the pulsation period was not noticeably varied. There was, however, a marked irregularity of rhythm. Long stops between strokes were noticed in some specimens, with a subsequent recovery and an acceleration of the heart rhythm. Animals thus treated with 1 per cent alcohol often lived for several hours after immersion in the alcohol. The heart of an animal in which the ganglion was removed stopped beating within five minutes after immersion in a solution of 1 per cent alcohol. In such an experiment, the alcohol undoubtedly reaches the heart tissues directly by way of the cut area. With a solution of 1 to 1000 of alcohol the normal heart is accelerated so as to beat from four to eight strokes per minute faster than the normal. In specimens in which the ganglion was removed, the same strength of alcohol gave an immediate depression in the rate of beating. Irregularity and a shortening of the pulsation period also occurred in some of the specimens.

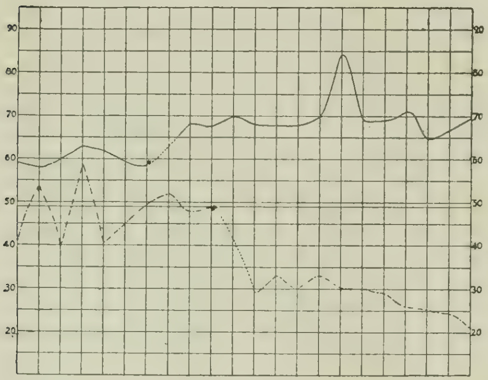

FIGURE 1. — The upper curve is from a normal animal; mean, at 60; the lower from an animal with ganglion removed; mean, about 51; * marks 1 : 1000 alcohol.

Normal animals, when placed in a 1 to 10,000 or 1 to 100,000 solution, failed to show any marked symptoms. In one or two cases a slight acceleration was noticed where animals were placed in a 1–10,000 solution; but as this soon became normal, the change of rhythm might be attributed to the shock caused by the changing of the fluids in the container. No results were obtained with operated specimens, the weak strengths of alcohol seemingly having no effect on them.

The present unsettled views as to the real nature of the influence exerted by alcohol, both on nerve and muscle tissue, makes the above notes of some interest. These experiments seem to fit in with the most recent evidence taken from the higher vertebrates, and with some of the findings in the invertebrate groups. (See Cushney,[1] Herter,[2] Kraepelin,[3] and others.) Fig. 1 shows graphically the effects of certain strengths of alcohol on normal and ganglionless animals.

Muscarin. — The well-known effects of muscarin sulphate, and the previous results obtained with it by Kruckenburg on Salpa, and by Lingle on Molgula, render these experiments hardly worthy of repetition. In the experiments of both of the above-mentioned investigators, muscarin had the characteristic depressant effect on the heart.

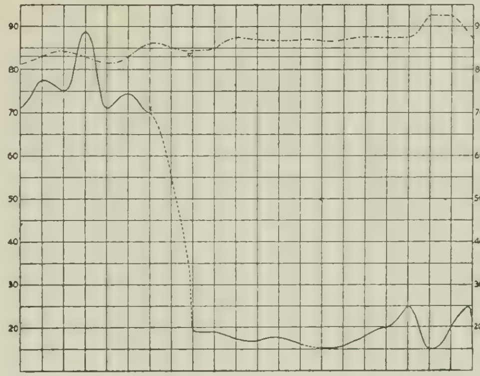

FIGURE 2. — The upper curve is from an animal with ganglion removed; mean, about 83; the lower curve is from a normal animal; mean, 75; * marks 1 : 1000 muscarin.

Only a few experiments could be made by the writer, owing to the difficulty of obtaining pure muscarin. Experiments with one set of solutions of muscarin turned out successfully; all the others failed. Either because of the poor quality of the drug obtained, or because of the extremely small quantity of muscarin present, solutions of 1–100,000 strength of muscarin sulphate had no effect, either on normal or ganglionless animals.

With a strength of 1–10,000 and 1–1000, the effects were immediate and marked. These effects were: (1) depression of the heart-beat; (2) increase in the length of the rest period between the pulsation periods; (3) increased irregularity in the heart rhythm. The ultimate effect, especially and immediately noticeable after the use of a 1–1000 solution, was the death of the animal. Frequently death was immediate, or within fifteen minutes after the addition of the

[1] CUSHNEY: Pharmacology, 1900.

[2] HERTER: Chemical pathology, 1902.

[3] KRAEPELIN: *vide* HERTER's Chemical pathology, 1902.

poison. More frequently, especially with a solution of 1–10,000, life continued for four, five, or even nine hours after the addition of the drug to the sea-water.

EXPERIMENT X[12].

August 25, 1902. — Specimen size, 16 × 18 mm. Fresh. Temperature of water, 21°.

Abvisceral.				Advisceral.		
Time of obs.	Beats.	Time.	Rest.	Beats.	Time.	Rest.
		min. sec.			min. sec.	
10.20 A. M.	67	1 47	2	53	1 35	1
	59	1 33	3	67	1 46	2
	63	1 28	2	55	1 22	2
	52[1]	1 29	1	44	1 10	3
	Added 1.1000 muscarin sulphate at 10.40 A. M.					
	48	1 16	1	35	1 1	4
	41	1 7	1	40	1 10	7
	41	1 13	1	36	1 2	6
	43	1 13	2	37	1 14	6
	41	1 12	2	38	1 19	5
	44	1 20	1	46	1 21	8
	43	1 20	1	47	1 22	8
	43	1 22	1	43	1 12	7
	45	1 23	0	41	1 20	5
	41	1 18	2	45[1]	1 20	10
11.13 A. M.	39	1 19	1	49	1 25	10
12.19 P. M.	39	1 15	1	52	1 20	3
	43	1 19	2	48	1 21	4
	36	1 16	1	60	1 41	3

[1] Irregular.

Fig. 2 shows graphically the depressant effect of muscarin sulphate on the normal heart of Molgula manhattensis. It is noticeable

that in some cases, at least, the advisceral pulsation appears to be depressed to a much greater degree than is the abvisceral beat. In one case, however, the opposite result was noted, *i.e.* the greater depression of the abvisceral beat.

The net results of several experiments show a depression which may be immediate, but which has a maximum several hours after the addition of the poison. One example shows an immediate depression of from six to eight beats per minute, and a lengthening of the rest period of the abvisceral pulse. This experiment is given in the table on page 16.

Another experiment shows a depression from the normal of fifty-five beats per one hundred seconds, fifteen minutes after the application of muscarin of 1–1000 strength. Other cases give a depression of from thirty to fifty-five beats per one hundred seconds, two and one-half to three hours after putting the animals in a solution of 1–1000 strength. In one case (1–10,000 strength) there is a depression of twenty-five beats per one hundred seconds, two and three-fourths hours after immersion in the poison. In nearly every specimen under the influence of muscarin, an irregularity amounting almost to a fluttering pulse is seen. This irregularity also makes its appearance in the heart rhythm, as can be seen in the irregularly longer rest periods between single beats in a given pulsation period.

A comparison of the results thus obtained with those reached by the immersion of the ganglionless animal in weak solutions of muscarin are instructive. In the case of extirpation of the ganglion or of the removal of the heart from the body, the influence of the drug appears to be slight. Instead of an inhibition we find an actual slight acceleration in some cases. The heart is very irregular. In no observed instance was death immediate. A typical experiment is the following:

Extirpated ganglion at 8.05 P.M., Sept. 1, 1902. 8.15 P.M., heart beating at the following rate per minute: 24, 26, 31, 31, 32, 36, 36, heart very irregular, beating from both ab- and advisceral ends at once. Added 1-1000 strength solution of muscarin sulphate at 8.23 P.M. At 8.25 P.M. heart beating very irregularly, mostly in an abvisceral direction, at the following rate: 38, 37, 37, 36, 38, 33, 37, 36, (reversal); 34. During the above beating the heart appeared to be accelerated, making the strokes very quickly; but with long stops between the series of beats in a given direction. 9.01–9.09 P.M., heart beating irregularly, mostly in advisceral direction, at the following rate: 24, 27, 30, 32, 31, 35, 37. In the above

series every twenty-fifth to thirtieth stroke there appeared a stronger beat which interrupted the advisceral beat, but which was quickly overcome by it, after which beating in the advisceral direction was resumed, until interrupted again about a minute later by this one strong beat in the abvisceral direction.

Comparison of these results with those of the investigators mentioned previously as workers on the Tunicata, with those of Ransom and Yung on the Mollusca, with those of Dogiel and Yung on the Crustacea, and finally with the many experiments on vertebrate material quoted by Gaskell,[1] shows the generally depressant effect of the drug. (Yung, however, finds acceleration preceding depression in certain of the Mollusca.) There has existed a difference of opinion as to the exact action of the drug in its depressant action on the heart. Ransom and some of the older writers favored action on the heart muscle; the later writers believe the drug acts through the nervous system. The fact that muscarin is ineffectual on the embryonic vertebrate heart, in which the nerve centres have not yet appeared, seems conclusive proof of the latter view. If the drug acts on the preganglionic fibre at the point of its connection with the nerve cell, as Gaskell believes is true for the Vertebrates, then the above-mentioned results might be taken to indicate the same state of affairs in Molgula. As heart ganglia and endings on heart-muscle have been found, together with connective fibres, the anatomical connection of which have not yet been fully worked out, it looks as if the connection between the heart and the central nervous system did exist in the Molgulæ.

Nicotin. — Experiments have been performed on the Salpæ by Kruckenburg, Lahille, and Schultze. According to Kruckenburg, poisoning with hellebore and nicotin affects only the duration of the advisceral pulsation period, and consequently influences only the hypobranchial end of the heart. He believes that the change in direction of the contractions is brought about by connection with the ganglion. "The results of poisoning with hellebore and nicotin . . . appear to me to indicate that the reversal of contractions is reflex and is brought about through the ganglion." He was, however, unable to prove the existence of ganglia on the heart, or of connective fibres between the central nervous system and the heart. He finds that the particular effect of nicotin is to reduce the number of advisceral pulsations,

[1] *Vide* GASKELL's article in Textbook of physiology, edited by SCHÄFER, London, 1900.

while hellebore increases the number of advisceral pulsations. Schultze took up the investigation to verify Kruckenburg's results, and obtained different findings. He used solutions of 1–10,000, 1–25,000, and 1–100,000 of nicotin, and worked on the large Salpa africana-maxima. With a 1–10,000 solution he finds an almost immediate and strong reduction in both ab- and advisceral pulsation periods. The animal dies after one-half an hour's immersion in the poison. With 1–25,000 strength, practically the same result is obtained except that the animal lives for one and one-half hours. In both of the above experiments Schultze gets a decided depression of the heart-beat and great irregularity. With 1–100,000 strength his figures show a slight increase in the length of the pulsation period, together with a slight depression in an abvisceral and acceleration in the advisceral direction. After a little 1–25,000 solution is added to the above, the heart almost immediately shows the effect by greater irregularity, depression, and a shortening of the pulsation period.

In my own experiments three solutions were used: 1–1000, 1–10,000, and 1–100,000. With the first-named strength, an almost instantaneous effect was obtained. Upon addition of the nicotin, the heart was seen to change its direction at once; then, after from one to two minutes beating, which was characterized by great irregularity of rhythm and depression, death would ensue.

The following are typical experiments:

Specimen, size 18 × 20 mm. Fresh. Temperature of water, 21°. Time of observation, 11.30 A. M. Normal animal in sea-water.

Abvisceral.			Advisceral.		
Beats.	Time.	Rest.	Beats.	Time.	Rest.
	min. sec.			min. sec.	
42	1 20	1	48	1 32	4
46	1 38	1	53	1 42	6
58	1 45	2	59	1 42	3
11.40 A. M. Added 1.1000 nicotin. Heart beating in advisceral direction. After 10 to 12 beats, it reversed.					
			26	1 32	20
1	?	?	1	3 0	
1					
		No more beating observed.			

Specimen, 12 × 14. Fresh. Temperature of water, 21°.

Abvisceral.			Advisceral.		
Beats.	Time.	Rest.	Beats.	Time.	Rest.
	min. sec.		167	min. sec. 3 28	2
150	3 1	1	161	3 2	1
148	2 49	1			
Added 1.1000 nicotin.		Heart-beat both directions at once for 10 seconds.			
			1	0 2	20
2	0 ·3	1.10	1		
		No more beating.			

With a strength of 1–10,000, death usually occurred within half an hour, frequently more quickly. The characteristic changes caused by the stronger solution were observed here, but in a less marked degree. It is interesting to note that in both of the above series of experiments the pulsation periods are shortened greatly, as observed by Kruckenburg and Schultze, the abvisceral pulse being affected the more decidedly. With a 1–100,000 strength an immediate but very slight depression of the heart, and a marked irregularity of the rhythm, occurred. The length of the pulsation was seemingly unaffected.

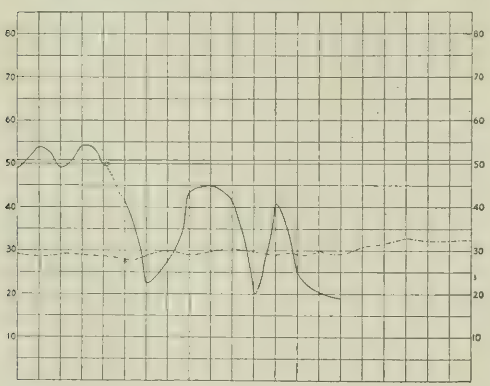

Figure 3. — The upper curve is from a normal animal; mean, about 51; the lower from an animal with ganglion removed; mean, 30; * marks 1 : 1000 nicotin.

Fig. 3 following gives a graphic view of the heart action when under the influence of 1–1000 and 1–10,000 nicotin. A comparison of the above results with those obtained with the use of Molgulæ in which the ganglion was extirpated is of interest. In the case of the strong solution (1–1000), the effect is somewhat like

that obtained in the unoperated Molgulæ with a solution of the same strength. The heart in all observed cases stopped beating within twenty-five minutes. The *immediate* depressant effect of the poison was not seen as in the normal animals, although present to a marked degree. The shortening of the pulsation period was not so marked as in the ganglionated animals. The characteristic abnormal irregularity of the heart rhythm, however, was very noticeable. In solutions of 1–10,000 and 1–100,000 strength, the depressant effect of nicotin is not observable, many cases showing an actual acceleration. Irregularity of rhythm is here characteristic, as in the other cited cases. A long rest period, irregular in time of appearance, is found between single beats. The example given below illustrates many of the experiments:

Ganglion removed 9.25 A. M., Sept. 3, 1902.

10.27 A. M. Heart beating in advisceral direction at the following rate per minute: 29, 29, 29, 29, 28, 29, 28.

10.35 A. M. Added 1–1000 nicotin.

10.36 A. M. Heart beating in advisceral direction at following rate: 30, 31, 31, 30, 30, 31.

10.52 A. M. Heart beating irregularly in both directions at once. Pulsations very firm. One or two stops of 3 to 5 seconds each. Rate: 30, 31, 31, 30, 30, 30.

11.30 A. M. Beating in advisceral direction; interrupted by a few beats in the opposite direction; pulsations then taking place in both directions at the same time. Rate: 32, 32, 34, 32, 34.

These experiments are of interest when considered in comparison with the well-known experiments of Langley on vertebrate material. If nicotin acts on the junction of the inhibitory fibre and the ganglion cells in the heart, we should expect to obtain the very results that were obtained in the above experiments on the operated Molgulæ, providing such an apparatus exists in the latter animal.

Strychnin. — According to Reid Hunt[1] the drug probably acts on certain parts of the nervous system (the vaso-motor centre especially), rather than on the heart itself in the vertebrates. In the frog, the heart is stimulated by small amounts of the poison, while large amounts weaken and retard it. In the invertebrates there seems to exist a divergence of opinion as to the action of the drug, and its meaning. Strychnin sulphate, according to Cushney, dissolves in a

[1] HUNT: Reference Handbook of the Medical Sciences, 1901, pp. 687–703.

little over 8000 parts of water. My experiments were made with solutions of 1–10,000, and 1–100,000. With a saturated solution in sea-water the effect was immediate. The heart was strongly depressed, there was loss of co-ordination, a shortening of the pulsation periods in both directions, and death within twenty-five to thirty minutes.

The following experiment is typical:

Specimen size, 12 × 16. Three days in aquarium. Temperature of water, 21°+.

	Abvisceral.			Advisceral.		
Time of obs.	Beats.	Time.	Rest.	Beats.	Time.	Rest.
	29	min. sec. 0 55	5	23	0 43	3
	23	0 57	5	13	0 38	2
	25	0 52	7	22	0 50	5
	28	0 55	6	30	0 56	3
	32	1 0	5	31	1 1	5
	36	1 5	5	34	1 6	5
	Added saturated solution of strychnin sulphate at 8.30 P.M.					
8.40–9.00 P.M.	....	....	....	44	1 20	5
	35	1 12	3	43	1 35	5
	33	1 21	4	27	1 35	2
	27	1 9	1	11	1 0	9
	8	0 33	2	6	0 30	?
	6	0 35	10	5	0 50	12
	1	0 10	....	1	0 20	
Beating at this time starts at one end of the heart and returns slowly.						
9.02 P. M. Slight contraction at abvisceral end of heart.						
9.04 P. M. Slight fibrillar contractions. No more movement observed.						

The results obtained with a 1–10,000 solution were practically the same as those recorded above, except that the effect is not so immediate or so marked.

Ganglionless Molgulæ, when put in a 1–10,000 solution, did not show an immediate depression of heart-beat, nor did death follow

immediately. (Unfortunately I was prevented, through lack of material, from trying the saturated solution on operated Molgulæ.)

With a 1–100,000 strength solution, the characteristic strychnin acceleration was obtained when working with normal Molgulæ. This acceleration was immediate and from seven to twelve beats per minute above the normal rate. In some cases, after ten to fifteen minutes had elapsed, irregularity in rhythm set in. This irregularity seemed especially marked in the advisceral beat, causing a decided depression. After one to two hours the rate of beating would become lowered so that it was below the normal. Along with this depression would appear great irregularity, both in the ab- and advisceral directions, but especially marked in the advisceral beat. In general, the advisceral end of the heart seemed more profoundly affected by the drug than the opposite end.

The tables on pp. 24 and 25 show the effect of strychnin sulphate, 1–100,000 solution, on a normal Molgula.

In Molgulæ in which the ganglion was removed the effect of a 1–100,000 solution was very slight, if, indeed, any effect could be noticed. In a few experiments a very slight depression was obtained, followed by a rise to the normal rate within a few minutes. This same effect might be produced by pouring water over the animals, and may be due to such an action. The graphic records showing the action of strychnin do not show the extreme depression and reduction in the advisceral direction observed in some animals. The results are, however, inserted for the sake of comparison.

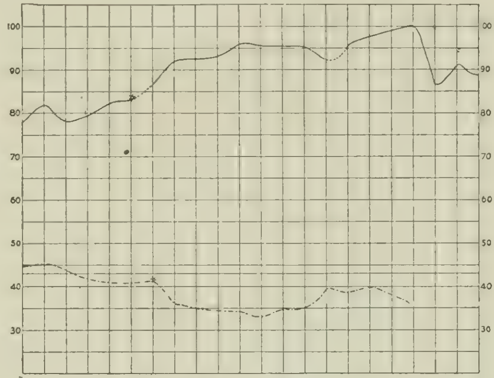

FIGURE 4. — The upper curve is from a normal animal; mean, 80; the lower from an animal with ganglion removed; mean, about 43; * marks 1 : 1000 strychnin.

Although results were obtained with the use of atropin, curari, and hellebore, it is not thought that these results have any direct bearing on the subject of acceleration or depression through the central nervous system, and so allusion to them, more than to say that, in a general way, they conform with experiments made on the vertebrates, will be omitted in this paper.

	Advisceral.			Abvisceral.		
Time of obs.	Beats.	Time.	Rest.	Beats.	Time.	Rest.
		min. sec.			min. sec.	
2.05–2.20 P.M.	47	1 10	4	56	1 36	4
	51	1 24	6	44	1 24	5
	48	1 26	7	46	1 22	4
	46	1 26	4	45	1 21	6
	50	1 28	4			
	Added 1.100,000 strychnin sulphate at 2.30 P. M.					
2.30 P.M.	....	....	....	65	1 40	4
	63	1 39	3	65	1 39	2
	64	1 38	2	55	1 22	3
	65	1 37	2	57	1 32	4
	67[1]	1 41	3	64	1 31	2
	69	1 30	2	58	1 29	3
	65	1 33	3	63[1]	1 23	3
	60	1 20	3	58[1]	1 18	4
	64	1 24	2	57	1 26	4
	52[1]	1 19	2	55	1 19	2
	62[1]	1 29	1	61	1 17	3
	65	1 29	1	59	1 19	1
	59	1 20	2	53	1 15	3
	58	1 20	2	50	1 19	4
	44	1 13	2	54	1 23	2
	52	1 20	5	53	1 18	2
	53[1]	1 11	8	41[1]	1 15	2
	51	1 15	8	41[1]	1 18	2
	50[1]	1 15	10	41[1]	1 24	1
	52[1]	1 14	10	26[1]	0 56	1
	47	1 12	8	36	1 6	4

[1] Irregular.

	Advisceral.			Abvisceral.		
Time of obs.	Beats.	Time.	Rest.	Beats.	Time.	Rest.
		min. sec.			min. sec.	
	45	1 20	8	27[1]	1 4	2
	45	1 24	10	19[1]	0 55	2
	41	1 13	7	20[1]	0 59	1
	47	1 15	7	18[1]	1 2	1
	43	1 13	7	17[1]	1 14	1
	43	1 15	10	13[1]	0 59	1
	44	?	13	1[1]	0 5	1
	36	1 4	20	2	0 20	20
	30	1 0	10	1	....	25
	35	0 57	20	1	....	30
4.02 P.M.	37	1 2	23	7	0 50	5
	40	1 15	20			
5.56 P.M.	....	....	....	1	0 5	
	2	0 10	....	7	0 40	
	35[1]	1 40	....	5	0 30	20
	2	?	10	5	?	
	30	1 10	50	15[1]	1 0	
8.06–8.12 P.M.	35[1]	1 35	....	12[1]	1 20	
	15[1]	0 40				
8.25 A.M.[2]	82[1]	3 20	3.15	1[3]	0 3	
	2	0 28	....	1[3]	0 3	15
	96[1]	3 51	4.38	1[3]	0 1	
	2	0 4	10	1[3]	0 1	

[1] Irregular.

[2] Sensory reflexes acute.

[3] A quick beat is rebuffed by another in the opposite direction (abvisceral) almost immediately.

Caffein and digitalin, drugs which are believed to act more or less directly on heart muscle, were also used in the series of experiments made on Molgula. The results obtained, although incomplete, will be given in part.

Caffein. — According to Bock,[1] caffein acts, in the vertebrates at least, chiefly on muscle. Acceleration takes place in the isolated heart. In the normal animal, however, inhibition may occur from the stimulation of the cardio-inhibitory centre.

A solution of 1–1000 strength of caffein was tried on the isolated heart of Molgula with the result that there was a slight but noticeable acceleration of the heart rhythm, and an increased regularity of the rhythm. The heart-beat seemed stronger and fuller than it was before the addition of the drug. Normal animals, when placed in a 1–1000 solution of caffein, showed an immediate depression of the heart-beat, which appeared within a few minutes (five to ten) after the addition of the drug, but which seemed less marked after perhaps half an hour. The general irritability of the muscle was much increased, and a decided irregularity of rhythm appeared, especially in the case of animals that had been some time in the solution. Results have not yet been obtained with solutions of less strength.

Digitalin. — According to Cushney,[2] who has made the most extensive researches with vertebrate material, the drug acts in two ways: *i.e.*, it first depresses the heart by acting on the inhibitory apparatus, and later accelerates the heart by its action on the heart muscle. This action he obtained with small doses of digitalin.

My experiments here are very incomplete, and I will only cite one or two. In animals deprived of the ganglion, and put in a 1–1000 solution of digitalin, the heart was accelerated and the rhythm rendered more regular.

The normal animal, when treated with the same strength solution, also showed a slight acceleration, but with accompanying irregularity of rhythm. There seems no doubt of the effect of the drug on the muscle in both of these experiments, because of a peculiar double heart-beat which appears and which seems to be characteristic of the digitalin stimulation of the muscle fibres.

[1] Bock: Archiv für experimentelle Pathologie und Pharmakologie, 1900, xliii, p. 397.

[2] Cushney: Journal of experimental medicine, 1897, ii, p. 254.

Conclusions.

The above results may be summarized as follows:

1. The normal heart beat of Molgula manhattensis varies greatly in different individuals as to rapidity of rhythm, duration of pulsation period, and rest period.

2. The average number of heart-beats in a given direction, either ab- or advisceral, was 43.2 per minute.

3. The length of the average rest period was two seconds.

4. In approximately 70 per cent of the animals examined the average number of heart-beats in a pulsation period in either direction did not exceed one hundred; in 30 per cent of the animals the pulsation period in a given direction exceeded one hundred beats, but rarely more than three hundred and fifty beats. Animals having a pulsation period of over three hundred and fifty to four hundred beats may safely be said to be abnormal in that respect.

5. In some observed cases the middle piece of an isolated heart of Molgula will beat when placed in sea-water; but in no cases did these pieces beat immediately after operation.

6. The removal of the posterior part of the ganglion (brain), or of the anterior end of the visceral nerve cord, affects the heart-beat of Molgula in the following respects: depression of the heart; irregularity of the heart rhythm (long pauses between beats, double beats, etc.); loss of co-ordination between the two ends of the heart; beating from both ends of the heart at the same time, either with or without co-ordinative rhythm; lengthening of the pulsation period in one direction to an abnormal degree, death not immediately following.

7. After treatment with certain specific nerve poisons, the heart of normal Molgulæ reacted in a different manner from those in which the ganglion had been removed.

8. Some specific muscle poisons affect the heart of normal and ganglionless animals in an almost identical manner.

In conclusion, the writer wishes to express his thanks to Prof. C. O. Whitman and Prof. F. R. Lillie for the privileges of an investigator's room at the Marine Biological Laboratory, Woods Hole, and to Prof. Jacques Loeb for many helpful suggestions. My thanks are also due to the members of the staff of the biological department of Columbia University for the courtesy shown me there.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. No. 143.

THE SKIN AND THE EYES AS RECEPTIVE ORGANS IN THE REACTIONS OF FROGS TO LIGHT.

BY G. H. PARKER.

INTRODUCTION.

ALTHOUGH the frog has probably served as the subject of more laboratory investigations than any other animal, its phototropism seems never to have excited more than passing comment. Graber ('84, p. 121) observed that when specimens of Rana esculenta were put in a box one half of which was illuminated and the other half in shadow, the animals were found about three times in the dark to twice in the light. This result might be interpreted to indicate that frogs are *negatively* phototropic, as, in fact, Loeb ('90, p. 89) subsequently intimated. Plateau ('89, p. 82), however, found that when specimens of Rana temporaria were liberated in a dark-box illuminated only by a pair of windows at one end, they jumped toward the windows. Thus R. temporaria seems to be *positively* phototropic.

In view of these somewhat contradictory statements, I attempted to determine whether our common leopard frog, Rana pipiens Schreber, is negatively or positively phototropic, and what parts are concerned as receptive organs in its reactions to light.

NORMAL FROGS.

Frogs upon which no operations had been performed were tested with Nernst lamps in a dark-room with blackened walls. These lamps possess the advantage of requiring no glass protection for their filaments, and thus reflections such as often cause great inconvenience in ordinary incandescent lamps are avoided. Moreover, their light in quality is much more nearly like daylight than that from incandescent lamps or the arc light. The Nernst lamps used were a "single glower" lamp heated by a current of 110 volts and a "six glower" lamp on a current of 220 volts. Heat was eliminated by

passing the light through a layer of seven centimetres of distilled water contained in a glass vessel with flat sides. With this heat screen attached, the one glower lamp had an apparent intensity of 24 to 25 candles. The six glower lamp under similar conditions gave a light of about 320 candle-power. These two lamps with their heat screens were the sources of light for all my experiments except where otherwise stated.

When frogs were put on a moist plate so that their long axes were at right angles to the direction of the rays of light, they sooner or later turned toward the source of light, and generally jumped in that direction. This occurred irrespective of the side of the frog that was exposed to the light, and was observed in animals that were 5 metres from the 25 candle-power lamp and consequently were in light of 1 candle-metre intensity, as well as with those that were 12.5 centimetres from the 320 candle-power lamp and were in light of an intensity of 20,480 candle-metres. Many intermediate intensities were tried and always with the same general results, namely, the frogs turned toward the source of light and usually jumped in that direction.

With the lower intensities the animals often did not react for from five to ten minutes or even longer, and the jumping response was frequently omitted; but their orientation was finally always with their heads toward the source of light, that is, positive. In some instance after a frog had remained ten minutes or more without changing its original position, it was induced to jump by being touched from behind, and, when this was done, the animal almost invariably turned first and then jumped toward the source of light. With high intensities, it was remarkable how persistently the frog would face the source of light and jump in that direction, even when the light was unbearably strong to the human eye.

From these experiments, I conclude that between the intensities of 1 and 20,480 candle-metres Rana pipiens is positively phototropic.

The Eyes.

It is natural to suppose that the positive phototropism of R. pipiens is dependent on the eyes; but it is conceivable that this phenomenon may depend upon the skin, or, since the tissues of the frog are more or less permeable to light, upon the direct stimulation of internal organs such as the brain and spinal cord.

To test the efficiency of the eyes in this respect, I attempted to eliminate the possible action of the skin and deeper parts by covering the animal, excepting the eyes, with opaque material. In my first trial I made a suit of clothes in one piece from soft light-proof cloth. This was slipped on the frog and held in place by a thread passed through the mouth like a horse's bit. The frog when thus covered remained motionless both on land and in water, its limbs taking unusual and often unsymmetrical positions, as though it were dead. On removing its covering, however, it immediately assumed a normal position and began jumping about. Apparently the cloth covering so stimulated it as to inhibit ordinary locomotion, and therefore I abandoned this form of experiment.

Frogs, even when closely crowded together, move continually, thus showing that the contact of the skin of one frog with that of another has no such inhibitory influence as the cloth exerted. I therefore killed a large dark-colored frog and removed its skin in one piece. This was turned inside out, thoroughly washed, and slipped over a somewhat smaller frog, on which it was held by a bit-like thread, as in the former experiment. The frog thus covered moved about with almost normal agility. The only portions of the living frog that were exposed were the front and hind feet, the snout, and the eyes. Four such animals were tested in light having an intensity of 50 candle-metres, and I found that these animals turned toward the light and jumped toward it much as normal frogs do. The experiment was made first with the covered frogs alone, but afterwards, for the sake of comparison, I introduced a normal frog into the receptacle each time I tested a covered one. In most instances the normal frog responded more quickly than the covered one, but the difference was not so great that it might not have been due to the purely mechanical interference of the covering skin.

It might be assumed, since the dead skin with which the frog was covered was not perfectly opaque, that even in these experiments the reactions really depended upon the influence of light on the skin; but this assumption is not warranted, for when the dead skin was drawn up over the eyes all evidence of phototropism disappeared. I therefore believe that I am entirely safe in concluding that Rana pipiens is positively phototropic to light stimuli received through the eyes.

The Skin.

Having found that the eyes were concerned in the phototropism of the frog, it remained to ascertain whether other parts acted as receptive organs for phototropic reflexes. To test the skin in this respect, I operated on frogs in the following way. By a single, vertical, transverse cut just behind the eyes, these organs and the cerebral hemispheres were removed with the snout of the animal. It is well known that frogs in this condition may with a little care be kept alive many weeks, and that the chief difference between these and normal frogs is the great reduction in spontaneous movements shown by the former. Frogs without cerebral hemispheres move, as a rule, only when stimulated by some obvious means.

I prepared in this way eleven frogs, and tested them in light of 50 candle-metres intensity. Of these, two never showed clear reactions to light, but the other nine were unmistakably phototropic. The following record of frog No. 5 will give a fair idea of the nature of these responses.

Frog No. 5. — Cerebral hemispheres and eyes removed May 1. Tested May 8.

4.35 P. M. The frog was placed with its *left* side toward the source of light. It soon began turning, a little at a time, directly toward the light, till at

4.48 P. M. it was facing the light.

4.50 P. M. The frog was placed with its *right* side toward the light. It soon began turning toward the light and at

4.54 P. M. it was facing the light.

4.55 P. M. It was again placed with its *left* side toward the light. By

5.02 P. M. it was facing the light.

5.03 P. M. It was placed with the head directly away from the light. It began turning to the right and at

5.16 P. M. it was facing the light.

May 9. At 1.35 P. M. the frog was placed with its head away from the light. By

1.46 P. M. it was facing the light and continued in this position till

2.38 P. M., when it jumped toward the light.

These records are fair samples of those obtained from the nine responsive frogs, and my observations on these have shown that the animals will turn by the shortest course either to the right or to the left toward a source of light, and, having obtained the position of positive

orientation, they will remain facing the light for a considerable period, usually terminated by a jump toward the light. In other words, eyeless frogs, like those with eyes, are *positively* phototropic.

These reactions are observable not only in artificial but also in natural light. Thus frog No. 5, on being placed with its left side toward a window through which bright diffuse daylight was entering, turned repeatedly, in from eight to twenty minutes, so that it faced the window. When, late in the afternoon, it was placed sidewise in sunlight it turned with every trial almost immediately toward the sun. Thus positive phototropism is observable in natural as well as in artificial light.

It is strange that the positive phototropism of eyeless frogs has not already been recorded, for in the majority of individuals it was found strikingly characteristic. Moleschott and Fubini ('79), in their numerous experiments on the influence of light on the excretion of carbon dioxide from frogs with and without eyes, must frequently have had animals under observation that should have shown this phenomenon, and yet they make no mention of it. Possibly the European species may differ in this respect from the American; but however this may be, there can be no doubt that positive phototropism is a characteristic not only of the normal Rana pipiens, but also of its eyeless conditions. In this respect R. pipiens resembles certain planarians, which, as Loeb ('94, p. 225) first observed, and Parker and Burnett (:00) subsequently worked out in detail, are phototropic both with and without eyes.

Having found that eyeless frogs were usually positively phototropic, it remained to ascertain what were the receptive organs in this reaction. Since in my first experiments I worked with frogs in which the optic lobes were somewhat exposed to light, it might be suspected that these organs received the stimulus directly. I therefore prepared other frogs in such a way that after the removal of the eyes, etc., a fold of skin was left to cover the exposed portion of the brain, and thus protect it from light. With this precaution, however, the frogs still continued positively phototropic. But it might still be supposed that the small amount of light which penetrated the tissues of the frog might reach the central nervous organs and act as an orienting stimulus. To test this possibility, I observed the effects of illuminating only a part of the frog's body. When an eyeless frog is placed in light of 50 candle-metres intensity, and about half of the skin on the exposed side is kept in shadow, the positive reactions often fail

to appear. If the obstruction is gradually removed, there comes a time before complete illumination when the animal will orient positively. By placing a screen in an appropriate position, it was possible to throw a shadow on that part of the frog's body which contained the central nervous organs, and still leave the greater part of the skin illuminated. Under such conditions the frogs in the great majority of cases turned toward the light, showing that the nerve endings in the skin were stimulated. Since no response was obtained from frogs that were covered with the skin of another frog so cut as to admit light to the region next the brain and spinal cord, I believe that these deep-seated organs are not only not essential as receptive organs in phototropism, but that they are not stimulated by such light as may reach them. I therefore conclude that the positive phototropism of eyeless frogs depends upon the capacity of the nervous structures of the frog's skin to be stimulated by light.

That the skins of some animals are normally open to stimulation by light has long been known. Willem ('91, p. 338), who twelve years ago prepared a résumé of this subject, enumerated some thirty-five species of metazoa in which this had been demonstrated, and Nagel ('96) subsequently added considerably to this number. Among the animals enumerated by Willem there is only a single representative of the vertebrates, Triton cristatus. This animal was studied by Graber ('84, p. 96), who prepared young individuals for experimentation by removing their eyes and then covering their heads with a layer of black wax. Animals thus prepared were put in a chamber part of which was illuminated and part in shade. In a total of 2102 observations the animals were found 674 times in the light and 1428 times in the shade. Graber, therefore, concluded that the skin of this newt could be stimulated by light.

Recently Beer (:01, p. 30) has called attention to a second species of vertebrate in which like conditions occur. This is Proteus anguineus, which, according to Configliachi and Rusconi ('19), becomes restless on sudden illumination and retreats eventually to the darkest situation it can find. This sensitiveness, which was also noticed by Semper ('81, p. 79), was attributed by Configliachi and Rusconi not so much to the stimulating effect of the light on the eyes, as to its influence on the skin. But the eyes of Proteus though very rudimentary, have been shown by Kohl ('95, p. 207) to possess all the essential parts of a functional organ of vision, and the reactions observed by Configliachi and Rusconi, so far as the evidence

advanced by them is concerned, may be explained entirely on the assumption that the rudimentary eyes are the receptive organs.

Dubois ('90, p. 358), however, has experimented on Proteus with much more conclusive results, for he has shown that it will respond to a small beam of light thrown on its tail. Moreover, when the eyes are covered with gelatine and lampblack, the animal will still respond to light. There is then good reason to suppose that the skin of Proteus is a receptive organ for light.

Finally Korányi ('93) has shown that, under conditions of exceptional excitability, a beam of strong light, when thrown on a frog's back, will induce reflex movements in the legs. It is thus evident that several amphibians, including the frog, possess skins with end-organs, sensitive to light.

The observations of Eigenmann (:00, p. 113) on the reactions to light of the blind-fishes Chologastre and Amblyopsis indicate that the nerve-endings in the skins of fishes also may be stimulated by light. I know of no evidence, however, that the skins of air-inhabiting vertebrates are ever thus normally stimulated. It is of course well known that when strong light is concentrated by a lens it may become a powerful stimulus for the nerve terminals in the human skin, but the organs that are affected by this are temperature organs and not organs of sight. Nevertheless it must be borne in mind that, as Korányi ('93, p. 7) has demonstrated, the skin of a frog is stimulated both by radiant heat and by light, and that these two influences, distinct as they seem to our senses, are members of one physical series in that they are both ether vibrations, varying only in wave lengths. It is therefore conceivable that in the lower vertebrates, like the frog, the end-organs in the skin are stimulated by radiant energy of a wide range, including what is for us both radiant heat and light, and that the descendants of these organs in the skins of the higher vertebrates are more restricted in function and are ordinarily sensitive only to radiant heat and its effects. It is thus possible that the temperature sense organs in the skins of the higher vertebrates may be specialized derivatives of radiant energy organs such as presumably occur in the skin of amphibians.

SUMMARY.

1. Rana pipiens is positively phototropic to light varying in intensity from 1 to 20,480 candle-metres. This applies always to the orientation and usually to the locomotion of the frog.

2. Individuals with the skin covered and the eyes exposed are positively phototropic.

3. Individuals with the eyes removed and the skin exposed are also as a rule positively phototropic.

4. The receptive organs in the phototropism of the frog are the eyes and the skin, but not the central nervous organs.

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INFLUENCE OF RENNIN UPON THE DIGESTION OF THE PROTEID CONSTITUENTS OF MILK.

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CONTENTS.

I. Introductory.

AFTER considerable investigating, Schumberg,[1] in 1884, concluded that the gastric juice of the adult contains relatively much more rennin than that of the infant, but made no attempt to establish a ratio. Later Szydlowski[2] investigated the rennin content of the gastric juice of infants, but because of his failure to neutralize the acidity of the stomach contents the work has not the value it otherwise would possess. As the result of experiments made in 1890, Du Saar[3] reported the absence of rennin in the gastric juice of infants.

Very recently (1900), Sternberg[4] has attempted to compare the rennin content of the gastric juice of infants and adults. One and one-half hours after the ingestion of an ordinary meal, it was his custom to remove the stomach contents for examination. Under these conditions it was observed that the gastric juice of the infant contained much less rennin than that of the adult. The former,

[1] Schumberg: Archiv für pathologische Anatomie, 1884, xcvii, p. 260.

[2] Szydlowski: Prager medicinische Wochenschrift, 1892, p. 365.

[3] Du Saar: Inaugural Dissertation, Amsterdam, 1890; Jahresbericht für Thierchemie, 1891, xxi, p. 252.

[4] Sternberg: Archiv für Physiologie, 1900, p. 362.

however, in opposition to the statement of Du Saar[1] showed a moderate rennin activity. Since the natural food of the infant is almost exclusively milk, Sternberg argued that the low rennin content in the gastric juice of infants would seem to indicate that rennin does not materially promote the digestion of the milk proteids. He then performed a series of artificial digestion experiments. A typical experiment was as follows:

100 c.c. of skimmed milk containing 0.56 gm. of nitrogen was treated with 0.32 gm. of pepsin containing 0.01 gm. of nitrogen, and the whole mixture thoroughly shaken. The milk was then divided into two portions. One half was treated with 0.02 gm. of rennin containing 0.002 gm. of nitrogen, and after the beginning of coagulation this portion, as well as the portion to which no rennin had been added, was treated with 100 c.c. of 0.3 per cent hydrochloric acid. The two portions were then placed on a water bath at 38° C., for fifteen minutes, neutralized with equal amounts of alkali, then treated with 0.262 gm. of trypsin containing 0.022 gm. of nitrogen, and left at 38° C. for two and one-half hours. At the end of this period the residues were filtered off and weighed in the customary manner. Of the 0.307 gm. of nitrogen in the portion to which no rennin had been added, only 0.012 gm. of nitrogen was recovered; whereas of the 0.309 gm. of nitrogen in the portion coagulated by rennin, 0.031 gm. of nitrogen was regained.

One purpose of the present work was to repeat Sternberg's experiments[2] by a somewhat different method, in an attempt to verify his conclusions. A number of control experiments and comparative tests to show the influence of rennin upon the digestion of fluid egg-albumen were made.[3]

II. Experimental.

1. Influence of rennin upon the gastric digestion of milk proteids. — In each of the eleven series of artificial digestion experiments following, freshly skimmed milk, secured immediately preceding the commencement of the experiments, was used.

[1] Du Saar: *Loc. cit.*

[2] Since the completion of this investigation in 1901, Popper (Archiv für die gesammte Physiologie) has reported the results of some experiments which, to a degree, support the conclusions I have drawn from my data.

[3] My thanks are due Professor Chittenden, for timely suggestions during the preparation of the data, and to Professor Mendel, under whose direction the experiments were made. I also wish to express my appreciation of the services of Mr. R. D. Milner.

The standard pepsin-hydrochloric acid solution used was prepared as follows: One and one-half gms. of commercial scale pepsin was dissolved in one litre of 0.3 per cent hydrochloric acid, and filtered.

The rennin was secured by the use of a commercial preparation of rennet. This was always carefully filtered, and a portion tested for coagulable material. The results in this direction were negative.

In all experiments ordinary digestion flasks were used. The length of the digestion period was variable; the temperature in no case was allowed to rise above 40° C.

TABLE I.

GASTRIC DIGESTION RESIDUES.

No.	I.	I a.	II.	III a.	IV.	IV a.	V.	VII.	VIII.
1	52.1	48.5	46.5	52.5	85.4	89.8	93.9	81.8	79.3
2	35.6	40.7	35.9	59.9	83.0	75.4	83.5	96.4	95.7
3	34.5	41.7	36.8	53.3	86.1	91.4	75.4	66.4	65.2
4	100.0	100.0	100.0	100.0	91.6	95.0	57.8	....	95.8
5	....	....	....	....	82.2	74.5	59.9		
6	....	....	....	....	100.0	100.0	100.0		

Series I. — The following experiments were made:

1. 10 c.c. milk, 1 c.c. rennet, 11 c.c. pepsin-hydrochloric acid.
2. 10 c.c. milk, 1 c.c. boiled rennet, 11 c.c. pepsin-hydrochloric acid.
3. 10 c.c. milk, 1 c.c. water, 11 c.c. pepsin-hydrochloric acid.
4. 10 c.c. milk, 1 c.c. water, 11 c.c. pepsin-hydrochloric acid (boiled).

After the rennet had been added to the skimmed milk, and the clot formed in Experiment 1, the pepsin-hydrochloric acid was added in each case, and digestion continued at 40° C. for one hour. At the end of this time the mixtures were heated to boiling to stop the digestion. The residues were next filtered on weighed papers, dried to constant weight at 110° C., and weights accurately determined. Weights of the residues[1] follow (gm.):

1	2	3	4
0.2820	0.1930	0.1870	0.5418

[1] In Table I, the weights have been tabulated so as to show in each series the percentage relation between the digestion residues and the absolute control. The absolute control is represented by 100.

Series Ia. — Experiments and conditions similar to those of Series I obtained here, except that after the rennet and milk were mixed in Experiment 1, the flask was vigorously shaken for some time in order to prevent the milk clotting in a solid mass.

Weights follow:

1	2	3	4
0.2734	0.2294	0.2352	0.5642

The results obtained in Series I and I a strongly indicate that the rennet has exercised an *inhibitory* action upon the digestion of the milk.

Series II. — In this series the pepsin-hydrochloric acid solution was not used, the pepsin and hydrochloric acid being added separately. The pepsin solution was made by dissolving 0.1 gm. of scale pepsin in 5 c.c. of distilled water.

The clot was allowed to form in Experiment 1 before the acid was added in any of the four experiments. Slight clotting occurred also in Experiments 2 and 3, due, no doubt, to the presence of rennin in the scale pepsin preparation. Digestion continued one hour and fifteen minutes.

Experiments and weights follow:

1. 10 c.c. milk, 1 c.c. rennet, 1 c.c. pepsin solution, 12 c.c. 0.3 per cent hydrochloric acid.
2. 10 c.c. milk, 1 c.c. boiled rennet, 1 c.c. pepsin solution, 12 c.c. 0.3 per cent hydrochloric acid.
3. 10 c.c. milk, 1 c.c. water, 1 c.c. pepsin solution, 12 c.c. 0.3 per cent hydrochloric acid.
4. 10 c.c. milk, 2 c.c. water, 12 c.c. 0.3 per cent hydrochloric acid.

Weights:

1	2	3	4
0.1986	0.1534	0.1574	0.4275

Series III. — It was now thought advisable to determine the influence of the *rennet ash* upon the digestion of the milk. To secure the "2 c.c. rennet ash" used in this series, 2 c.c. of the commercial rennet was evaporated carefully, and the solid residue burned to an ash. This ash, dissolved in 2 c.c. distilled water, was then used in the experiment.

Data follow:

1. 10 c.c. milk, "2 c.c. rennet ash," 12 c.c. pepsin-hydrochloric acid.
2. 10 c.c. milk, 2 c.c. rennet, 12 c.c. pepsin-hydrochloric acid.
3. 10 c.c. milk, 2 c.c. boiled rennet, 12 c.c. pepsin-hydrochloric acid.

Digestion continued forty-five minutes.

Weights:

1	2	3
0.1196	0.2034	0.1560

Because of an unexpected, exceedingly rapid digestion in Experiment 1, the series was repeated.

Series IIIa. — This series included three experiments similar to those of Series III, supplemented by a fourth as follows:

4. 10 c c. milk, 2 c.c. boiled rennet, 12 c.c. pepsin-hydrochloric acid (boiled).

After digesting one-half hour, the following weights were obtained:

1	2	3	4
0.1728	0.1974	0.1756	0.3294

It will be noticed that the boiled rennet and the rennet ash give approximately the same result, indicating that the source of the inhibitory action of rennin upon the digestion of milk proteids is not to be sought in the ash.

Series IV. — A pepsin solution prepared as follows was used in the experiments of this and following series: 0.06 gm. of scale pepsin was dissolved in 20 c.c. of distilled water, thus giving 0.003 gm. of pepsin to 1 c.c. of the solution.

Experiments were as follows:

1. 1 c.c. rennet, 1 c.c. pepsin solution, 10 c.c. milk, 12 c.c. 0.3 per cent hydrochloric acid.
2. 1 c.c. boiled rennet, 1 c.c. pepsin solution, 10 c.c. milk, 12 c.c. 0.3 per cent hydrochloric acid.
3. 1 c.c. rennet, 10 c.c. milk, 1 c.c. pepsin solution, 12 c.c. 0.3 per cent hydrochloric acid.
4. 1 c.c. rennet, 10 c.c. milk, 1 c.c. pepsin solution (boiled), 12 c.c. 0.3 per cent hydrochloric acid.
5. 1 c.c. boiled rennet, 10 c.c. milk, 1 c.c. pepsin solution, 12 c.c. 0.3 per cent hydrochloric acid.
6. 1 c.c. boiled rennet, 1 c.c. pepsin solution (boiled), 10 c.c. milk, 12 c.c. 0.3 per cent hydrochloric acid.

The first two materials in each experiment were combined and allowed to act, at 40° C., for one-half hour before the digestion mixture was completed, the purpose of thus combining the materials in different order being to observe any possible action of the pepsin upon the rennin.

Weights:

1	2	3	4	5	6
0.3056	0.2968	0.3082	0.3278	0.2942	0.3578

As the short period of digestion (one-half hour) did not bring out the variations sufficiently, this series was repeated, and the digestion continued for one hour:

Series IVa. — The experiments in this series were similar to those of Series IV, the time of digestion however being increased to one hour.

Weights :

1	2	3	4	5	6
0.2930	0.2461	0.2984	0.3102	0.2430	0.3264

The results of Series IV and IV a seem to indicate that the inhibitory action of rennin upon the digestion of milk proteids is not modified by preliminary contact with the pepsin solution at 40° C. for one-half hour.

Series V. — In Experiments 1 and 2, the rennet was added to the milk, and as soon as the clot formed, the temperature of the mixture was brought to the boiling point for a few moments. It was noticed that a compact mass was formed which could not be broken up with a glass rod. The conditions would no doubt have been more satisfactory had the mixture been thoroughly stirred before boiling. In Experiment 3, the clot was formed as in Experiments 1 and 2, but the contents of the flask were not subjected to the boiling temperature. In Experiment 4, the boiled rennet and milk were combined, and the mixture brought to a boil. When these steps were completed, the flasks were cooled, the remaining ingredients added to each, and the digestion proper begun and continued one hour.

Experiments :

1. 20 c.c. milk, 1 c.c. rennet (clotted and boiled, and 1 c.c. rennet and 20 c.c. pepsin-hydrochloric acid added).
2. 20 c.c. milk, 1 c.c. rennet (clotted and boiled, and 1 c.c. water and 20 c.c. pepsin-hydrochloric acid added).
3. 20 c.c. milk, 1 c.c. rennet (clotted, and 1 c.c. water and 20 c.c. pepsin-hydrochloric acid added).
4. 20 c.c. milk, 1 c.c. boiled rennet (boiled, and 1 c.c. water and 20 c.c. pepsin-hydrochloric acid added).
5. 20 c.c. milk, 2 c.c. water, 20 c.c. pepsin-hydrochloric acid.
6. 20 c.c. milk, 2 c.c. water, 20 c.c. pepsin-hydrochloric acid (boiled).

Weights follow :

1	2	3	4	5	6
0.7222	0.6418	0.5798	0.4448	0.4608	0.7690

Series VI. — In preparation for the experiments of this series, casein was prepared from one pint of skimmed milk, by precipitation by hydrochloric acid. The crude product was washed free from hydrochloric acid with water, then subsequently washed with alcohol, dried, and ground.

Paracasein was also prepared from one pint of the same lot of milk, by treatment with rennet solution. The crude paracasein was then filtered off and washed, dried, and ground, as above.

Experiments on paracasein :

1. 1 gm. paracasein, 20 c.c. pepsin-hydrochloric acid.
2. 1 gm. paracasein, 20 c.c. pepsin-hydrochloric acid.

Experiments on casein :

3. 1 gm. casein, 20 c.c. pepsin-hydrochloric acid.
4. 1 gm. casein, 20 c.c. pepsin-hydrochloric acid.

The solid matter in Experiments 3 and 4 entirely disappeared in forty-five minutes. In Experiments 1 and 2 the digestion was less rapid. No weights were taken, but the series was repeated, using larger amounts.

Series VII. — For these experiments casein was prepared from one quart of skimmed milk, and paracasein from a like amount. Methods of preparation were the same as those previously used.

Experiments on paracasein :

1. 12 gms. paracasein, 50 c.c. pepsin-hydrochloric acid.
2. 12 gms. paracasein, 50 c.c. pepsin-hydrochloric acid (boiled).

Experiments on casein :

3. 12 gms. casein, 50 c.c. pepsin-hydrochloric acid.
4. 12 gms. casein, 50 c.c. pepsin-hydrochloric acid (boiled).

50 c.c. was found to be too small an amount of fluid, and an additional 50 c.c. of 0.3 per cent hydrochloric acid was added to each experiment at the end of an hour.

Weights :

1	2	3	4
9.8202	11.5670	7.9628	(lost)

Series VIII. — Experiments were similar to those of Series VII.

Weights follow :

1	2	3	4
9.5214	11.4810	7.8212	11.5013

From the results of Series VII and VIII it is evident that the casein has been digested to a slightly greater extent than the paracasein.

2. **Influence of rennin upon the pancreatic digestion of milk proteids.** — Four series of artificial digestion experiments were made in this connection. The preparation of pancreatic digestion fluid was as follows: 20 gms. of dry pancreas was treated with 200 c.c. of 0.1 per cent salicylic acid and placed in a thermostat for twelve hours. The solution was then strained and made alkaline with 0.5 per cent sodium carbonate, after which it was thymolized vigorously and its activity upon fibrin tested. Satisfactory activity was indicated.

Series I. — Experiments were as follows :

1. 10 c.c. milk, 1 c.c. rennet, 11 c.c. tryptic solution.
2. 10 c.c. milk, 1 c.c. boiled rennet, 11 c.c. tryptic solution.
3. 10 c.c. milk, 1 c.c. water, 11 c.c. tryptic solution.
4. 10 c.c. milk, 1 c.c. water, 11 c.c. tryptic solution (boiled).

After the addition of rennet in Experiment 1 the contents of the flask were vigorously shaken to prevent clotting in a solid mass. In order to stop the action of the trypsin previous to drying and weighing the residues, the contents of the flasks were treated with equal amounts of acid and heated.

Weights:[1]

1	2	3	4
0.1666	0.1212	0.1140	0.4484

Series II. — All conditions and experiments the same as in Series I.

Weights:

1	2	3	4
0.1790	0.1310	0.1206	0.4728

From the results of Series I and II we may draw the conclusion that the digestion of milk proteids in an *alkaline* tryptic solution is retarded by the presence of rennin.

TABLE II.

PANCREATIC DIGESTION RESIDUES.

No.	I.	II.	III.	IV.
1	37.2	37.9	34.4	35.3
2	27.0	27.7	24.5	23.9
3	25.4	25.5	24.2	23.8
4	100.0	100.0	100.0	100.0

Series III. — In Series III and IV the tryptic solution was made *neutral* before using. Preliminary trials were unsatisfactory, owing to the fact that a too active tryptic solution was used, and that the quantity of milk was too small. The resulting residues being exceedingly light, unavoidable errors in weights, etc., however minute, would seriously alter the result and prevent the drawing of accurate conclusions from data so obtained. Hence the series was repeated with a less active tryptic solution and with the use of a larger amount of milk. The results obtained under these conditions are given in Series III and IV.

Experiments:

1. 20 c.c. milk, 1 c.c. rennet, 11 c.c. tryptic solution.
2. 20 c.c. milk, 1 c.c. boiled rennet, 11 c.c. tryptic solution.
3. 20 c.c. milk, 1 c.c. water, 11 c.c. tryptic solution.
4. 20 c.c. milk, 1 c.c. water, 11 c.c. tryptic solution (boiled).

[1] See Table II.

Weights:

1.	2	3	4
0.2416	0.1723	0.1697	0.7024

To stop the action of the trypsin, the digestion mixtures were treated with acid and heated as in Series I.

Series IV. — Conditions and experiments similar to those of Series III.

Weights:

1	2	3	4
0.2520	0.1706	0.1701	0.7145

The experiments of Series III and IV in neutral solution indicate that the rennin has exerted a retardation similar to that shown in alkaline solution.

3. **Comparative tests upon fluid egg-albumen.** — In these tests three series of gastric digestion experiments were made. The preparation of fluid egg-albumen was as follows:[1] The undiluted whites of a large number of eggs were treated with hydrochloric acid having a specific gravity of 1.12 (4.2 c.c. hydrochloric acid to 300 c.c. white), and shaken vigorously. After standing some hours, the solution was filtered to remove globulin, and the filtrate made exactly neutral with 0.5 per cent sodium carbonate. The albumen thus prepared contained a trifle less than 2 gms. of coagulable proteid in 20 c.c.

Series I. — Following experiments were made:

1. 20 c.c. albumen, 2 c.c. rennet, 22 c.c. pepsin-hydrochloric acid.
2. 20 c.c. albumen, 2 c.c. boiled rennet, 22 c.c. pepsin-hydrochloric acid.
3. 20 c.c. albumen, 2 c.c. water, 22 c.c. pepsin-hydrochloric acid.
4. 20 c.c. albumen, 2 c.c. water, 22 c.c. pepsin-hydrochloric acid (boiled).

These digestion mixtures were found to be too concentrated. The proteid and hydrochloric acid united to form *combined* hydrochloric acid, leaving no *free* acid present. No weights were made.

Series II. — Conditions were the same here as in Series I, except that 30 c.c. of distilled water was used to dilute the 20 c.c. of egg-albumen in each case, and 52 c.c. of pepsin-hydrochloric acid added, making a total volume of 104 c.c. These were digested for five hours.

Experiments:

1. 20 c.c. albumen, 30 c.c. water, 2 c.c. rennet, 52 c.c. pepsin-hydrochloric acid.
2. 20 c.c. albumen, 30 c.c. water, 2 c.c. boiled rennet, 52 c.c. pepsin-hydrochloric acid
3. 20 c.c. albumen, 32 c.c. water, 52 c.c. pepsin-hydrochloric acid.
4. 20 c.c. albumen, 32 c.c. water, 52 c.c. pepsin-hydrochloric acid (boiled).

[1] Schütz: Zeitschrift für physiologische Chemie, 1885, ix, p. 581; Chittenden and Mendel: American journal of the medical sciences, 1896.

Weights:[1]

1	2	3	4
0.4354	0.4436	0.4314	2.7204

TABLE III.

Egg-Albumen Residues (Gastric Digestion).

No.	II.	III.
1	16.0	16.2
2	16 3	16.0
3	15.9	16.1
4	100.0	100.0

Series III. — All conditions and experiments similar to those of Series II. Weights follow:

1	2	3	4
0.4436	0.4390	0.4420	2.7416

From the results of Series II and III we may conclude that rennin has no inhibitory action upon the digestion of fluid egg-albumen.

III. Conclusions.

1. Rennin inhibits the gastric digestion of milk proteids.
2. Rennin ash does not possess this inhibitory action.
3. The inhibitory action of rennin upon the digestion of milk proteids is not modified by preliminary contact with pepsin solution at 40° C. for one-half hour.
4. Paracasein is somewhat more difficult of digestion than casein.
5. Rennin retards the pancreatic digestion of milk proteids in alkaline or neutral solution.
6. Rennin has no inhibitory action upon the gastric digestion of fluid egg-albumen.

[1] See Table III.

RESPIRATION EXPERIMENTS IN PHLORHIZIN DIABETES.

By ARTHUR R. MANDEL and GRAHAM LUSK.

[*From the Physiological Laboratory of the University and Bellevue Hospital Medical College.*]

THE literature concerning this subject has already been presented in Voit's Festschrift,[1] where a preliminary experiment is described. Only a brief summary is, therefore, permissible. The laws of nutrition in mammals demand a certain calorific production for a square metre of surface area. This heat may be derived from proteid, fat, or carbohydrates.

In 1867, Pettenkofer and Voit[2] compared the metabolism of a diabetic with that of a normal man. The authors showed that a diabetic on a mixed diet consumed a larger quantity of proteid and fat than a normal man who was able to burn sugar. The following example of their work may be cited :

	Healthy Man.			Diabetic Man.		
	Proteid.	Fat.	Sugar.	Proteid.	Fat.	Sugar.[1]
Nutriment . . .	120	112	344	107	108	337
Burned in the body	120	83	344	158	192	0

[1] 337 gms. in the urine.

It is clear that more fat and more proteid have been burned in the diabetic than in the healthy man, in order to supply the calories lost through the sugar. Several years later, on the basis of these experiments, Erwin Voit[3] calculated that, on a moderate mixed diet, the normal man burned food of a heat value of 1,026 calories per

[1] Lusk: Zeitschrift für Biologie, 1901, xlii, p. 31.

[2] Pettenkofer und Voit: Zeitschrift für Biologie, 1867, iii, p. 380.

[3] Lusk: Zeitschrift für Biologiè, 1890, xxvii, p. 478.

square metre of surface, while a diabetic of similar build gave off 1,015 calories.

The experiments of Reilly, Nolan, and Lusk[1] showed that after producing the diabetic condition with phlorhizin in fasting dogs, the proteid metabolism may rise as much as 560 per cent, as a result of the non-burning of the sugar. This alone would give sufficient heat to replace that lost through the non-burning of the sugar. It therefore seemed possible that the starving diabetic burned no more fat than the normal animal in starvation. This hypothesis was further strengthened by the fact that in starvation the blood becomes rich in fat, which is carried to the tissues for combustion, and that the fat metabolism in inanition cannot be increased by feeding additional fat. The fasting liver, free from glycogen, accumulates fat.[2] The fasting organism is therefore plentifully supplied with fat from the reserve store of that material present in its own body. In diabetes, the condition produced by the lack of carbohydrate becomes still further aggravated. The sugar-hungry cells of the diabetic attract fat in larger quantity than they can burn it, and the blood-plasma may even become milk-white, due to the transportation of fat particles. This fatty infiltration of the cells is the result of their carbohydrate hunger.

An analogous state of affairs exists in phosphorus poisoning.[3] The proteid combustion is abnormal, and is higher than usual. The combustion of sugar from proteid is probably curtailed, and other products, as leucin, tyrosin, and lactic acid, are heaped up in the organism. The fatty infiltration described by Rosenfeld again results from the sugar-hunger of the cells.

The question before us is, does the diabetic burn more fat than the normal organism under similar dietary conditions, or does the increased proteid combustion supply the additional power necessary for his organism?

In a former paper, Lusk[4] has described a preliminary experiment in which he compared the metabolism of a fasting dog with that of the same dog rendered diabetic with phlorhizin; the result of his work is as follows:

[1] Reilly, Nolan, and Lusk: This journal, 1898, i, p. 395.
[2] Rosenfeld: Ergebnisse der Physiologie, 1902, i, p. 672.
[3] Ray, McDermott, and Lusk: This journal, 1899, iii, p. 139.
[4] Lusk: Zeitschrift für Biologie, 1901, xlii, p. 37.

	Normal Dog.			Diabetic Dog.[1]		
	Proteid.	Fat.	Total.	Proteid.	Fat.	Total.
Burned in the body	20.19	55.87	....	67.38	51.15	....
Calories	80.68	526.13	606.81	124.08	481.69	605.77

[1] 39.4 gms. dextrose in urine.

On the diabetic day the ratio between dextrose and nitrogen in the urine was 3.65 : 1. The details of the experiment are given in the article referred to.

The results of the above experiment led Lusk to conclude that in diabetes the calorific energy lost through the urine in the form of sugar was made up, not by increased combustion of fat, but by the rise in proteid metabolism.

We desired to confirm this result, and also to note the metabolism in the diabetic when meat and fat were fed. Large quantities of fat are necessary to the diabetic; but is the quantity more than that required by an Esquimaux who lives on bear-meat and whale-oil ?

The Experiments.

The method of the experimentation was similar to that described in Lusk's article in Voit's Festschrift.

The animal was kept for twelve and once for twenty-four hours in Voit's small respiration apparatus, a copy of which is in the laboratory. The dog was removed from time to time for catheterization and for the injection of phlorhizin. Nitrogen was determined by the Kjeldahl, dextrose by the Allihn method.

The carbon in the urine of the normal fasting dog was calculated according to Rubner[1] (1 gm. N = 0.728 gm. C, in fasting urine). The same author's figures were used for the computation of the carbon in the urine of the meat-feeding days (1 gm. N = 0.610 gm. C, in meat urine). As Pettenkofer and Voit[2] have shown that the diabetic urine contains in addition to this only the carbon of the sugar, we have added the dextrose carbon on the diabetic days.

[1] Rubner: Zeitschrift für Biologie, 1885, xxi, p. 329.

[2] Pettenkofer and Voit: *Loc. cit.*, p. 404.

We have not calculated the carbon and nitrogen of the fæces. In fasting this would make little difference, and besides this Rubner[1] has shown that the fæcal N and C are in such a proportion that they scarcely influence the calculation of fat.

The N in the urine was multiplied by 3.29 in order to determine the carbon from proteid. Rubner's calorimetric values were also accepted.

1 gm. N in fasting	= 24.98 calories.
1 gm. N in meat feeding	= 25.98 calories.
1 gm. C from fat	= 12.31 calories.

Stohmann gives 3.692 calories as the heat value of 1 gm. of dextrose. Assuming that 3.65 gms. of dextrose appear in the urine to one of nitrogen, we can calculate a loss of 13.47 calories (3.65×3.692). Therefore the calorific value of 1 gm. of N in diabetes will be $24.98 - 13.47 = 11.51$ calories for the fasting urine or 12.51 calories for the urine after feeding meat.

The D : N ratio of 3.65 : 1 was present in Lusk's former experiment, and the general average in the two respiration experiments we have completed do not vary widely from this. The record in Dog I is as follows:

DOG I.

Date. 1903.	Period.	N.	D.	D : N.	Phlorhizin every 8 hrs.	Remarks.
Feb. 22	I	3.864	24.16	6.25	gms. 5	
22	II					
23	I	7.01	27.61	3.93	5	
23	II	7.35	25.43	3.46		
24	I	13.05	49.87	3.81	2	300 gms. meat.
24	II	7.95	24.50	3.08		
25	I	11.28	45.94	4.06	2	300 gms. meat + 30 gms. fat.
25	II					
26	I	6.64	21.00	3.16	1	

[1] RUBNER: *Loc. cit.*, p. 323.

During all the Periods I the dog was in the respiration apparatus. These figures are not reduced to exactly twelve-hour periods as they will be later in this paper.

On February 23 the ratios average 3.69 : 1, and on the meat-feeding day 3.55 : 1. The higher ratio in Periods I after feeding meat simply means an earlier elimination of the proteid sugar than of the nitrogen belonging to it; the low ratio of 3.16 : 1, on February 26, the only one where the calculated heat value for proteid cannot be used with approximate accuracy.

In the second experiment the following results were obtained:

DOG II.

Date. 1903.	Period.	Weight.	N.	D.	D : N.	Phlorhizin.
		kg.				
Apr. 18	..	18.2				
20	..	17.6	2.07			
21	..	....	....	....	....	9.30 A.M., 5 gms. 3.00 P.M., 5 gms. 11.00 P.M., 3 gms.
22	I	17.1	7.31	27.87	3.78	9.00 A.M., 3 gms. 4.00 P.M., 1 gm.
22	II	....	7.14	23.74	3.34	11.00 P.M., 1 gm.
23	I, II	16.5	12.81	46.27	3.61	9.00 A.M., 1 gm. 3.40 P.M., 1 gm. 9.00 P.M., 1 gm. 3.14 A.M., 1 gm.
24	..	15.8				

On April 22, in the first period, the ratio was slightly higher than 3.65. In the second period it was lower. The average is 3.57 : 1, or approximately normal. The explanation is simple. At night there was an interval of ten hours between the injections, and the maximal effect of the phlorhizin was not present to completely remove the sugar, which, however, is not burned but is stored and eliminated on a renewal of the phlorhizin. For the discussion of this, see Stiles and Lusk.[1]

Our ratios in these experiments lead us, therefore, to accept a heat value of proteid in diabetes based on a sugar elimination of 3.65 gms. to one of nitrogen.

The fate of the phlorhizin carbon. — Lusk, in Voit's Festschrift,[2]

[1] Stiles and Lusk: This journal, 1903, x, p. 67.

[2] Lusk: *Loc. cit.*, p. 39.

noted that the fate of the phlorhizin carbon was of great importance in the proper calculation of the results in the respiration experiments. After citing the literature, he described the occurrence of a crystalline sediment of phlorhizin in the urine of a cat previously treated with phlorhizin.

If ordinary diabetic urine contains the normal proportion of carbon belonging to the nitrogen eliminated plus the carbon of the sugar, without any other carbon, and if the phlorhizin carbon is eliminated in the urine, then any "extra carbon" should be proportional to the phlorhizin given.

We have done two experiments. Dr. Mandel proved the accuracy of his combustion of the urine by doing several preliminary experiments with sugar alone, with urine alone, and with a known mixture of both together. The combustions were made in duplicate, after evaporating the urine in a platinum boat.

The first dog fasted for three days, and then received 5 gms. of phlorhizin every eight hours for two days. On the next day 5 gms. of phlorhizin were injected subcutaneously, and the urine was collected in two subsequent four-hour periods, and analyzed. N–C represents the carbon in the urine usually eliminated with the nitrogen found ($N \times 0.728$). D–C is the amount of dextrose carbon.

DOG A.

Weight = 12.0 kg.

Period.	D.	N.	D : N.	Phlorhizin. C.	N–C.	D–C.	Total C. (Calc.)	C found in urine.	Extra C.
I	7.81	2.50	3.12	2.73	1.82	3.13	4.95	6.03	1.08
II	6.59	2.48	2.66	....	1.80	2.64	4.44	5.27	0.83

In this dog the D : N ratio was on a 2.8 : 1 basis. The animal had been used in a previous experiment. During the first four hours after the injection of 5 gms. of phlorhizin, more extra carbon came out than during the second four. Of 2.73 gms. injected, 1.91 gms. may have appeared in the urine.

A second experiment was done on another dog. The animal fasted two days. He then received 2 gms. phlorhizin every eight hours for one day, and 2 gms. once and 5 gms. twice on the second day. The urine was then collected in eight-hour periods. During Periods

I, III, and IV, 5 gms. of phlorhizin were subcutaneously injected at the beginning of the period, and during Periods II and V, one-tenth of a gram was injected every two hours. The results are as follows:

DOG B.

Weight = 17.5 kg.

Period.	D.	N.	Phlor-hizin. C.	N–C.	D–C.	Total C. (Calc.)	C found in urine.	Extra C.
I	22.62	6.65	2.73	4.83	9.05	13.88	15.11	1.23
II	25.25	6.72	0.22	4.90	10.10	15.00	15.34	0.34
III	21.89	6.21	2.73	5.41	8.76	13.27	14.98	1.70
IV	....	....	2.73					
V	23.70	6.84	0.22	4.98	9.48	14.46	15.28	0.72

In dogs A and B we see that the injection of 5 gms. of phlorhizin (2.73 gms. C) leads to the elimination of 1.91, 1.23, and 1.70 gms. of extra carbon in the urine. The injection of 0.4 gm. of phlorhizin (0.22 gm. C) leads to the elimination of 0.34 and 0.72 gms. of such extra carbon. The figures represent an elimination of approximately 60 per cent of the phlorhizin carbon in the urine after administering 5 gms. subcutaneously. It is possible that some phlorhizin carbon is excreted through the intestine, but assuming 40 per cent of it passes off in the respiration, then in Dog I on February 23 to 24 there might have been an elimination of 1.68 gms. of carbon from phlorhizin in the twelve hours respiration. In none of the other periods could the phlorhizin carbon have appreciably affected the results.

It is clear that, in the earlier days of phlorhizin diabetes, the carbon in the urine is made up of that normally excreted with the nitrogen plus the carbon of the sugar plus an amount proportional to the quantity of phlorhizin administered. Since the "extra carbon" is very small when very small quantities of phlorhizin are given, there can be no appreciable elimination of oxybutyric acid.

We may now consider the results of the respiration experiments upon the dogs whose diabetic condition we have described.

Dog I. — February 20 was the fourth starvation day. On February 21, the total metabolism was calculated as 371 calories for the twelve-hour period. On February 22, diabetes was produced through injec-

tion of phlorhizin in 5 gm. doses. The calories produced are calculated on the assumption that none of the body's sugar was burned. It is quite possible, however, that some sugar was burned in the organism for a time after this first injection. In that case the 347 calories calculated may have been lower than those actually produced. On the second day of diabetes (the seventh of starvation), the total calories remain at about the simple starvation level. Less fat is burned, while the heat from proteid makes good this loss and the loss from the non-combustion of the proteid sugar. The proteid metabo-

DOG I.

Weight, February 20, = 14.2 kg. Periods of 12 hrs.

Date. 1903.	N.	C in urine.	C in resp.	Total C.	C of fat.	Heat. Proteid.	Heat. Fat.	Heat. Total.	Remarks.
Feb. 20	1.85	1.35	32.21	33.56	27.46	46.2	338.0	384.2	
21	1.53	1.12	30.96	32.08	27.03	38.3	332.7	371.0	
22	3.83	8.39	28.93	37.32	24.60	44.2	302.8	347.0	Diabetes.
23	7.00	15.24	29.96	45.20	22.29	80.2	274.4	354.6	
24	12.94	26.75	37.05	63.83	21.26	161.9	261.7	423.6	300 gms. meat.
25	11.22	23.21	38.78	61.96	25.15	140.4	309.5	449.9	300 gms. meat + 30 gms. fat.
26	6.64	14.53	33.78	48.30	26.45	76.5	325.6	402.1	

lism has risen 457 per cent above that in starvation. On February 24, 300 gms. of meat were fed. The fat combustion falls slightly with the simultaneous rise in the heat available from proteid. The total metabolism increases, — a necessary consequence of the additional stimulus of the organism through food, and of intestinal activity (Darmarbeit). Rubner[1] notices a rise from 243.5 calories to 329.9 calories after feeding a normal 5 kg. dog which had fasted five days with 390 gms. of meat. On February 25, 300 gms. of meat and 30 gms. of lard were fed. The total metabolism still rises. The fat feeding apparently delayed the meat absorption, since the nitrogen for the twelve hours is less than that of the day before. The fat combustion rises, but not above the combustion on February 20 and 21, which were days of simple starvation. This amount of meat,

[1] RUBNER: Zeitschrift für Biologie, 1894, xxx, p. 122.

containing 10.2 gms. of nitrogen and 37.54 gms. of carbon, with fat containing 22.95 gms. of carbon (total carbon = 60.48 gms.)[1] barely contained sufficient material for a nitrogen and carbon equilibrium for the twelve-hour period. Notwithstanding this, on the following starvation day there is an increased combustion of fat accompanying a decrease in the proteid metabolism. But the fat combustion does not exceed that of the days of simple inanition. The total metabolism does not return at once to the low level previous to the meat feeding.

We have, in the above, another experiment confirming the proposition that the fat metabolism in diabetes does not rise above that in the normal organism.

Dog II. — We have done one experiment which does not accord with the above statement. There is an apparent rise of 16 per cent in the total metabolism on comparing the fasting with the diabetic day. The experiment loses somewhat in authority because it has not the record of two fasting days.

On April 20 and April 22, the dog was in the respiration apparatus for twelve hours. On April 23–24, he was there for twenty-three hours of the twenty-four. The whole is calculated to a twenty-four-hour basis.

DOG II.

April 20, weight = 17.6 kg. Periods of 24 hrs.

Date. 1903.	N.	C in urine.	C in resp.	Total C.	C of fat.	Heat. Proteid.	Heat. Fat.	Heat. Total.	Remarks.
Apr. 20	4.14	3.02	60.80	63.82	50.20	103.42	617.98	721.38	
22	15.50	31.76	70 22	101.98	54.28	166.90	668.18	835.08	Diabetes.
23	12.75	27.89	67.26	95.15	53.20	144.75	656.12	800.87	50 gms. meat + 100 gms. fat.

For three days, up to and including the morning of April 18, the dog had been richly fed on meat and fat. He was in excellent condition. The first experimental day was the second of fasting. On April 22, the second diabetic day, there was a rise in proteid metabolism of 375 per cent. The calculated heat production rises 16 per cent and the fat metabolism 8 per cent. This was an unexpected result and does not agree with our other work. Rubner[2]

[1] Calculated according to Rubner: *Loc. cit.*, p. 126.

[2] Rubner: *Loc. cit.*, p. 120.

notices a variation in total calories in a starving dog, but not as great as in the above case.

On April 23, 50 gms. of meat and 100 gms. of lard were fed. There was no diarrhœa, and the fæces the next day had the normal appearance of pitch-like slime. The small amount of meat (= 1.7 gm. N) failed to increase the nitrogen output in the urine, and its effect may therefore be neglected; while feeding 100 gms. of fat failed in any way to increase the combustion of that material during the twenty-four hours the animal was under observation in the respiration apparatus. This experiment shows that feeding fat to a starving diabetic in good condition will not increase the fat metabolism.

Conclusions.

In general, the results of our work lead us to the following conclusions:

1. The calories lost in the urinary sugar in diabetes are compensated for in the increased proteid metabolism.

2. In a diabetic dog, whether he be fasting, or fed on meat alone, or on fat alone, or on meat and fat together, no more fat is burned than in the same dog when he is normal and fasting.

3. After the injection of 5 gms. of phlorhizin subcutaneously, as much as 60 per cent of the phlorhizin carbon may be eliminated in the urine.

4. In the early stages of diabetes due to phlorhizin, the carbon in the urine derived from oxybutyric acid or other abnormal products, except sugar (and phlorhizin itself), appears to be negligible.

THE EFFECT OF LECITHIN ON THE GROWTH OF THE WHITE RAT.

By SHINKISHI HATAI.

[*From the Neurological Laboratory of the University of Chicago.*]

IN 1895 Danielewsky found that frog eggs placed in water containing $\frac{1}{1500}$ by weight of lecithin gained in fifty-four days 300 per cent more in weight than those reared in ordinary water. His experiment has been repeated by a large number of investigators, who not only have confirmed the statement made by him for the frog's egg, but also noticed a similar physiological effect of lecithin on the growth of much higher animals.

Very recently Desgrey and Zaky ('00–'02), who studied the effect of lecithin on the animal body by examining the constitution of the urine, as well as determining the weight of the nervous and skeletal systems in relation to the total body-weight, published a series of observations in which they offered an explanation of the more rapid growth, as well as the probable fate of the lecithin after it has been taken into the living organism. In these papers they advance the opinion that the beneficial effect of the lecithin in the living body is that of a stimulating agent.

Since such observations have an important practical bearing, it is desirable to test the previous results repeatedly, as well as to investigate the matter from various sides, by using different animals. The object of the present investigation was to determine whether corresponding results could be obtained by applying the lecithin to animals not heretofore studied, and especially to examine the condition of the nervous system of the experimental animals in more detail.

The following experiments were made on five different groups of white rats. The first three groups were injected with lecithin subcutaneously, while the fourth and fifth were fed with lecithin through the mouth. The body-weights, as well as the sexes of individual animals, are given under each experiment. The lecithin used was prepared from the sheep's brain by Dr. Koch of the pharmacological laboratory of the University of Chicago. The technique used by

him for the isolation of the substance is given in detail in his paper published in 1902. Each rat in the first three groups was injected once each day with 8 minims of aqueous solution, which contained 0.005 gm. of the lecithin; while each rat in the fourth and fifth groups was fed with the same amount of the solution, which, however, contained 0.01 gm. of the substance. The doses given for the present investigation are proportional to those given by Desgrey and Zaky to the guinea-pig.

The lecithin emulsion in distilled water was boiled, in order to render it sterile, before it was used. In each case, the control and experimented rats were maintained under like conditions by keeping them in the same cage. They were fed at the same time with the same kind of food. A very plain vegetable diet was given, such as corn, carrots, cabbage, etc. Rich food, like meat, cheese, milk, etc., was avoided. The body-weights of the animals, both control and experimented, were determined at intervals of every two days, just before feeding. The food was given about half an hour after the administration of the lecithin. The injection was made with a fine syringe, and the feeding was done by means of a glass pipette.

The methods employed for a determination of water and solids in the nervous system, and the technique used for a microscopical examination of the peripheral nerves, will be given under the corresponding paragraphs.

Series I contains one male rat having a body-weight of 29.75 gms. for the control, and one male having a body-weight of 29.15 for experiment. They were taken from the same litter. The lecithin rat received 0.005 gm. of lecithin daily. The injection was made subcutaneously once each day. The experiment continued for about a month (from July 15 to August 13). During this experiment, the room temperature was constant, and the animals were in a healthy condition. The following results were obtained: As Table I, page 60, shows, during a month the injected rat gained 30.71 gms. in body-weight, while the control rat gained 23.16 gms.; in other words, the lecithin rat gained 32 per cent more in weight than the control. The weight of the central nervous system is also heavier in the lecithin than in the control rat. The last relation will be discussed later on.

Series II contains one male rat for the control, and two males for experiment. They belonged to the same litter. The experimental rats received 0.005 gm. of lecithin daily, subcutaneously. The experiment continued from August 21 to September 18, and the following

results were obtained: The body-weights gained during this period are much smaller than in the case of Series I; the control animal gained 14.49 gms., while the lecithin animals gained 19.75 gms. on the average.

This slight gain of the body-weight is probably due to the fact that while this experiment was in progress, the temperature was very irregular, and the animals were unfavorably affected by it. Two important facts have been brought out by this experiment: (1) the gain in body-weight by the lecithin animals is greater than in the control rat; in the former, the gain in body-weights was 36.3 per cent in excess; (2) on comparing the records of daily increase of the body-weight, in both control and lecithin rats, it appears that the former is much more irregular than the latter, showing that the injected animals were less evidently affected by the unfavorable conditions. These two results just mentioned have been confirmed by later experiments.

Series III contains one male rat for the control, and one for experiment. They belonged to the same litter. The experimented animal received 0.005 gm. of lecithin daily. The experiment was carried through from September 30 to October 27. While this experiment was in progress, the temperature was extremely irregular, and, in addition, the building was filled with the smell of fresh paint. These two circumstances appeared to retard the growth of the animals considerably. At the end of the experiment the following results were obtained. The control rat gained 9.23 gms. in body-weight, while the experimented rat gained 9.62 gms., the difference between the amounts of gain being only 0.385 gm. or 4 per cent. The result obtained from the present experiment is an unsatisfactory one, for the difference in the body-weights gained by the lecithin rat over that of the control is only 4 per cent, contrasted with 32 per cent and 36.2 per cent in the former series. Judging from the other experiments, the result obtained from this series may be attributed to an abnormal physiological condition during the time of the experiment.

Series IV contains two male rats for the control, and two other males for experiment. In this series, the animals were fed daily with 0.01 gm. of lecithin. The experiment was carried through from September 29 to October 26. During this period, the temperature was very irregular, and, in addition, the anatomy building, where these animals were kept, was filled with the smell of fresh paint. These two circumstances seemed to retard growth. The following

results were obtained: The control rats gained, on the average, 2.44 gms. in the body-weight, while the experimented animals gained, on the average, 11.69 gms. In this series also the experimented animals gained in body-weight more rapidly than the controls, and furthermore the former proved more resistant to the unfavorable surroundings. The weight of the central nervous system of the experimented rats is slightly greater than that of the controls.

TABLE I.

Showing the Average Results obtained from the Five Different Series of Rats used for Experiment.

Series.	Sex.	Body-wt. at start.	Body-wt. at end.	Gain in body wt	Wt. of central nervous syst.	Percentage of water in central nervous system.
I.	Cont. m.	29.755	52.915	23.16	1.667	
	Exp. m.	29.155	59.865	30.71	1.816	
II.	Cont. m.	43.165	57.655	14.49	1.755	
	Exp. m.	39.555	59.255	19.75	1.745	
III.	Cont. m.	44.555	53.780	9.23	1.772	77.3
	Exp. m.	37.305	47.120	9.62	1.736	77.3
IV.	Cont. m.	34.058	36.495	2.44	1.584	77.0
	Exp. m.	32.440	44.130	11.69	1.698	76.8
V.	Cont. f.	27.808	33.930	6.12	1.586	76.2
	Exp. f.	32.770	52.130	19.36	1.739	76.7
Av.	Cont.	35.810	46.955	11.15	1.672	76.8
	Exp.	34.360	52.500	18.14	1.747	76.9

Relative development of sheath and axis in cross sections of the nerve fibres.				
	Sciatic.		Brachial.	
	Axis.	Sheath.	Axis.	Sheath.
Control.	−8%	+8%	−0.5%	+0.5%
Experiment.	−3%	+3%	−2.5%	+2.5%

Series V contains two female rats for the control and two females for experiment. They all belonged to the same litter. The experimental animals were fed with 0.01 gm. of lecithin daily. The experiment was made between September 29 and October 26, as in the case of Series IV. Owing to the irregular temperature and the smell of paint in the building, as in the case of the former Series III and IV, the animals gained in weight but slightly. The controls gained, on the

average, 6.12, and the experimented animals gained, on the average, 19.36 gms. The experimented animals gained in body-weight more rapidly than the controls, and, in addition, the record of the daily increase of the body-weight indicates that the animals which received the lecithin have grown more regularly than the controls, thus confirming the results of the former experiments. The weight of the central nervous system in the experimented animals is also slightly heavier than in the controls.

From the preceding experiments, it is clearly shown that the animals treated with lecithin grow more rapidly than those which did not receive it. An average figure, based on all the series taken together, shows that the weight gained by the former group is 60 per cent in excess of the gain made by controls. This augmentation of the body-weight in the experimented animals is especially notable when the surrounding conditions are unfavorable for growth. This appears to depend on the fact that the lecithin animals exhibit a greater power of resistance to unfavorable changes in the surrounding

TABLE Ia.

SHOWING THE AVERAGE WEIGHTS OF ALL THE CONTROL RATS AND ALL THE LECITHIN RATS COMBINED.

The data are used for the construction of the curves in the accompanying figure.

Day.	Control group.	Lecithin group.	Day.	Control group.	Lecithin group.
	grams.	grams.		grams.	grams.
1	35.8	34.4	17	43.2	46.0
3	36.0	35.7	19	45.7	46.2
5	37.8	37.3	21	46.4	47.3
7	38.9	38 8	23	45.4	47.9
9	39.6	39.9	25	47.7	47.5
11	39.7	41.2	27	49.0	50.7
13	42.0	42.5	29	46.9	52.5
15	42.2	43.5			

conditions. This is shown by comparing the records of the daily increase of the body-weight in both experimented and control animals. In the case of the former, the gain in weight is constant, while in the latter the amount of the gain fluctuates with the changes in the

weather. This fact just mentioned is clearly shown in Fig. 1, in which the curves illustrating the rate of the growth of the animals, both control and experimented, are given.

Desgrey and Zaky's investigation in regard to the effect of lecithin on the animal body shows also a more rapid increase of the body-weight of the experimented animals, as compared with that of the controls. The following table exhibits the results obtained by them and also by myself:

TABLE II.

Showing the Relative Growth Rate of the Control and Experimented Animals.

	Animals.	Body-weight gained.		Ratios between control and experiment.	Nature of experiment.	Period.
		Cont.	Exp.			
Desgrey and Zaky.	Guinea-pig	730	1062	1 : 1.49	Feeding	52 days
	"	543	692	1 : 1.27	Injection	44 "
	Rabbit	480	620	1 : 1.29	Feeding	57 "
	Dog	300	1380	1 : 4.60	Feeding	30 "
	"	1480	2050	1 : 1.38	Injection	37 "
Hatai.	Rat	4.278	15.52	1 : 3.70	Feeding	30 "
	"	16.98	21.26	1 : 1.25	Injection	30 "

As the above table shows, the results obtained by Desgrey and Zaky, and by the present writer, point in the same direction as the earlier observation of Danielewsky ('95), who showed that frog's larvæ, under the influence of lecithin, increase in body-weight more rapidly than those reared in ordinary water.

It is shown in Table II that the animals fed with lecithin gain in body-weight more rapidly than the injected animals. Desgrey and Zaky's observation is confirmed by my experiments. Just what produces such a difference between the two methods, the writer is unable to explain at the present moment. It is to be noted that in the case of the rats, the dose of lecithin was twice as large in the feeding experiments as in the injection experiments, yet to compensate for this there must have been a greater loss in administration of the lecithin by the mouth.

From Table I, it is shown that the central nervous system in the experimented animals is slightly heavier than in the controls. It is well known, however, that in the same species, the animal having a heavier body-weight has also the heavier nervous system. It is necessary to determine, therefore, in this connection, whether the greater weight of the central nervous system is normal, or whether it deviates from the normal. To determine this question rightly, a large number of observations showing the relative weight of the nervous system and the body in normally grown animals, is demanded. Such records are fortunately already available in this laboratory. Comparing with these data those obtained from the present experiment, it was shown that the weight of the central nervous system in the experimented animals falls within the limits of the variation of the brain-weights in the normally grown rats with a corresponding body-weight. This means that the experimented animals have a central nervous system proportional to the body-weight, and that the development of this system is to be regarded as normal.

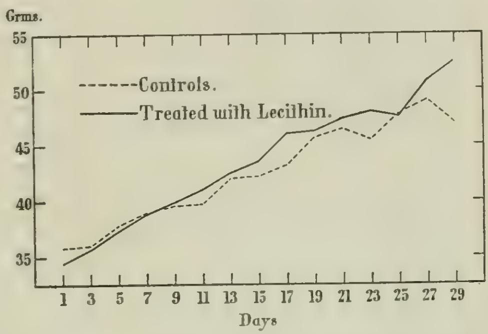

FIGURE 1. — Composite curves showing the body-weights of white rats at different ages.

Another question arises in this connection, namely, whether the nervous system in the experimented animal contains the same proportions of water and solids as in the control. It is important to determine this, since it has been shown in this laboratory that, as the animal grows older, the proportion of water in the central nervous system decreases. It appears, moreover, that the amount of water in the central nervous system is a function of age rather than of body-weight. If, then, the growth of the central nervous system in the experimented animals was normal, we should expect that the percentage of water would be the same as that found in the controls. In order to determine this question, the animals belonging to Series III, IV, and V were used, the central nervous systems were removed (brain and spinal cord, without roots), and their weights were determined in a fresh condition. They were kept in the steam bath for one week at a temperature of 90°, and then

their weights were determined again. From this experiment the following results were obtained:

TABLE III.

Showing the Amount of Water in the Nervous System.

		Cont.	Exp.
		per cent.	per cent.
III.	Per cent of water in the central nervous system, brain and cord combined . . .	77.3	77.3
IV.	Per cent of water in the central nervous system, brain and cord combined . . .	77.0	76.8
V.	Per cent of water in the central nervous system, brain and cord combined . . .	76.2	76.7
	Average per cent of water	76.8	76.9

From the above figures it may be concluded that the nervous system, in both control and experimented animals, contains the same proportional amount of water and solids, and the heavier weight of the central nervous system in the latter means more increase in the volume, without change in the proportional amount of the two constituents. The administration of lecithin, therefore, does not alter the proportion of water in the central nervous system of the animal receiving it. This result contradicts, in a measure, that obtained by Desgrey and Zaky, who found that the central nervous system of the experimented animals contained lecithin and phosporus slightly in excess of that found in the controls. A careful examination of the analytical tables given by these authors shows, however, that the excess in question does not always occur, since in another group of analyses the opposite result is obtained. Taking the average of all the cases, the amount of lecithin and phosphorus is found approximately equal in both the experimented and control animals, the slight differences which appear being probably due to individual variation and experimental errors combined.

It is also important to extend these observations to the peripheral nerves, with a view to determining the development of the medullary substance in that part of the nervous system. However, on account of the difficulty in collecting a sufficient quantity of the material for analysis, the examination was restricted to the determination of the relative areas of the medullary sheath and axis cylinder in the cross

sections of the peripheral nerves. Observation has already shown that in the normal rat, as in vertebrates in general, the area of the medullary sheath exposed in a cross section of the nerve fibres of the spinal roots, killed in osmic acid, is almost exactly equal to the area of the enclosed axis (data taken from a completed but unpublished research by Mr. G. W. Hoke).

For this purpose, small pieces of the sciatic and brachial nerves were removed and preserved in osmic acid (1 per cent) for twenty-four hours. The nerves thus preserved were imbedded in paraffin, according to the usual procedure, and were cut 6 micra in thickness. In each case, the areas of ten larger and ten smaller fibres were measured. It was assumed that in the ideal nerve fibres, the area of the medullary sheath would be exactly equal to that of the enclosed axis. Departure from the ideal relation could then be expressed as a percentage deviation, expressed as a plus quantity for the proportion in excess, and a minus quantity for the portion which was deficient. From this observation the following results were obtained:

TABLE IV.

Showing the Departure from the Ideal Relationship, expressed as a Percentage.

		Control.		Experiment.	
		Axis.	Medullary sheath.	Axis.	Medullary sheath.
		per cent.	per cent.	per cent.	per cent.
I.	In the fibres of the sciatic nerve	−8.0	+8.0[1]	−3.0	+3.0
II.	In the fibres of the brachial nerve	−0.5	+0.5	−2.5	+2.5

[1] The large deviation here is probably due to a stretching of the fibres during preparation.

The above figures indicate that the relative areas of the axis cylinder to the sheath are approximately the same in both control and experimented animals, and it may be concluded that the peripheral nerves also have grown proportionately in both groups.

From the facts given in the above, the writer concludes that: (1) the white rats which received the lecithin by either injection or feeding, gain in body-weight more rapidly than those which did not receive it, the gain in the experimented rats being on an average

60 per cent greater than in the controls; (2) the relative weight of the central nervous system in the lecithin rats was normal; (3) the nervous system in the experimented animals contains the same proportion of solids and water as in the controls; this is another indication of the normal character of the growth; (4) the relative area of the axis cylinder to its sheath in the nerve fibres of the experimented rats is approximately the same as that in the controls, showing that the peripheral nerves have also grown normally; (5) the rats which received the lecithin show a greater power of resistance to the unfavorable changes in the surroundings; (6) the present investigation confirms strongly the previous observation of Danielewsky, Desgrey, and Zaky, and others who claim the physiological effect of the lecithin to be that of a stimulating agent for normal growth.

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ON THE ACTION OF PHLORHIZIN.

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CREMER in the "Ergebnisse der Physiologie"[1] asks: "Is there a total phlorhizin diabetes?" In other words, is the influence of phlorhizin on the organism such that dextrose cannot be burned? Loewi[2] complains that his results with phlorhizin are very variable.

Four propositions will be discussed in this paper: (1) the constancy of the ratio between dextrose and nitrogen in the urine, (2) the quantitative eliminiation of dextrose fed *per os*, (3) the protection from combustion of sugar formed from proteid within the organism, and (4) the protection from combustion of dextrose administered subcutaneously.

The Constancy of the Ratio D : N in the Urine, and the Quantitative Eliminiation of Dextrose fed per Os.

It was first shown by Reilly, Nolan, and Lusk[3] that after a preliminary sweeping out of the body's sugar through the subcutaneous injection of 1 to 5 gms. of phlorhizin in a fasting dog every eight hours, there is established a definite ratio between urinary dextrose and nitrogen which approximates, — Dextrose : Nitrogen : : 3.75 : 1. Under ordinary circumstances this ratio is not changed after feeding meat or gelatin. Halsey[4] found the same ratio after feeding casein. Dextrose, up to 24 gms. fed *per os*, is also quantitatively eliminated. This last point was confirmed by Loewi,[5] who found an extra elimination of 8.5 gms. of dextrose after feeding 9 gms.

[1] CREMER: Ergebnisse der Physiologie, i, 1902, p. 883.

[2] LOEWI: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvii, p. 68.

[3] REILLY, NOLAN, and LUSK: This journal, 1898, i, p. 395.

[4] HALSEY: Sitzungsberichte der Gesellschaft zur Beförderung der gesammten Wissenschaften, Marburg, 1899, p. 102.

[5] LOEWI: *Ibid.*, 1901, p. 89.

These considerations led Lusk[1] to make the statement that in phlorhizin diabetes the dog's organism has lost the power to burn sugar. This statement was qualified by the observation that when sugar was fed in large quantity, it could probably be burned. This is indicated in the older experiments of Moritz and Prausnitz,[2] who found no increase in the proteid metabolism after the simultaneous administration of phlorhizin and 180 gms. of carbohydrates. The burning of the sugar fed prevented the customary rise in the nitrogen of the urine which accompanies the diabetic condition. Another proof of the ability of the phlorhizinized organism to burn sugar fed in excess, was shown by Lee and Harrold[3] in the increased ability to do work by a phlorhizinized cat's muscle, if carbohydrates had been fed in excess. There is, therefore, a "total phlorhizin diabetes" within certain limits, but these limits must not be overstepped by flooding the organism with sugar. In this latter case sugar can be burned.

The ratios obtained in Lusk's laboratory are more regular than those obtained in Loewi's work, and Loewi has sought to explain the differences by tabulating the animals used according to their weights. He claims that the larger dogs used in Lusk's laboratory give higher ratios than smaller dogs. He and his pupil Knopf[4] say that Halsey first pointed out the fact that the ratio could vary between 4.20 : 1 to 28 : 1. In making these statements they show a superficial knowledge of Lusk's work. In the first publication, and in others from his laboratory, Lusk has not only recorded but sought to explain such variations.

Fifty-six records taken from about fifteen dogs in this laboratory show the following D : N values on such fasting or meat feeding days, when there could have been no influence from carbohydrate feeding or other irregular influence (excessive meat feeding, for example).

5	between	4.00–4.44	11	between	3.70–3.60
	(three of these from one dog)		12	"	3.60–3.50
3	between	4.00–3.90	9	"	3.50–3.40
7	"	3.90–3.80	3	"	3.30–3.20
5	"	3.80–3.70	1	"	3.20–3.10

[1] Lusk: Zeitschrift für Biologie, 1901, xlii, p. 33.

[2] Moritz and Prausnitz: Zeitschrift für Biologie, 1890, xxvii, p. 118.

[3] Lee and Harrold: Proceedings of the American Physiological Society, This journal, 1900, iv, p. ix.

[4] Knopf: Archiv für experimentelle Pathologie und Pharmakologie, 1903, xlix, p. 123.

Besides this, the sugar elimination in three dogs fell to a 2.8 basis.

Eighty per cent of the above ratios vary between 3.89 and 3.40. Similar variations take place in individual dogs, as in the case of the first six experimented on by Reilly, Nolan, and Lusk.

	Highest.	Lowest.
Dog I	3.78	3.57
Dog II	3.91	3.54
Dog III	3.89	3.53
Dog IV	4.20	3.81
Dog V	3.71	3.41
Dog VI	3.86	3.42

What are the causes of the variability?

The very high ratio. We have found a permanently high ratio in one dog (Dog I, this article, p. 76), three portions of the urine yielding 4.06, 4.44, and 4.34. We cannot explain this exception to the general rule of our work, and we regard it as an abnormal result. The other two high ratios of 4.20 and 4.06 were of temporary character, and therefore of no especial significance.

The very low ratio. It has been stated that in three of our dogs the ratio fell to 2.8 : 1, the same as that observed in the rabbit,[1] the goat,[2] and the cat.[3] Loewi[4] and Knopf[5] do not even mention the fact that this ratio was commented on by Reilly, Nolan, and Lusk, who found it in dogs suffering from albuminuria and ascribed it to a pathological kidney. The production of artificial nephritis in rabbits with cantharidin or chromate may partially or completely abolish the susceptibility to phlorhizin.[6] Parker and Lusk[7] found

[1] LUSK: Zeitschrift für Biologie, 1898, xxxvii, p. 181.

[2] LUSK: Zeitschrift für Biologie, 1901, xlii, p. 41.

[3] ARTEAGA: This journal, 1901, iv, p. 103.

[4] LOEWI: *Loc. cit.*

[5] KNOPF: *Loc. cit.*

[6] HELLIN and SPIRO: Archiv für experimentelle Pathologie und Pharmakologie, 1896, xxxviii, p. 368, and RICHTER: Zeitschrift für klinische Medizin, 1900, xli, p. 160.

[7] PARKER and LUSK: This journal, 1900, iii, p. 472.

that if a rabbit were given a benzoate, the simultaneous injection of phlorhizin resulted in a D : N ratio lower than usual. Finally Jackson[1] observed that giving one dose of camphor to a phlorhizinized dog, with an established ratio of 3.75 : 1, resulted in the immediate fall of the ratio to a 2.8 basis. Loewi's[2] camphor experiments indicate this same relation. Many of Knopf's recent experiments were made on one dog whose ratio was at this lower level. He found a ratio of 2.8 : 1, whether 0.01 gm. or 1 gm. of phlorhizin was injected every eight hours (Table III). Possibly in the Marburg pharmacological laboratory it was not fully realized that dogs should be used whose kidneys have been unaffected by other drugs.

Lusk[3] has suggested an explanation of the difference between the 3.75 and the 2.8 ratios, by supposing that the intact phlorhizinized dog's kidney has the power of splitting a definite compound of dextrose formed in proteid metabolism, a compound which is normally burned in the animals where the 2.8 ratio prevails.

Subsequent to this explanation, Loewi[4] conceived the idea that all the sugar in the blood is normally held in a loose combination with colloid, which is broken up by the phlorhizin kidney.[5] We will return to the discussion of this problem later.

The medium ratios. — The four medium ratios between 3.10 and 3.40 can be explained by a partial retention of the sugar or by a failing kidney.

The influence of feeding fat. — Loewi[6] points out that the varia-

[1] JACKSON: Proceedings of the American Physiological Society, 1902. This journal, 1903, viii, p. xxxi.

[2] LOEWI: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvii, p. 56.

[3] LUSK: Zeitschrift für Biologie, 1901, xlii, p. 33.

[4] LOEWI: Archiv für experimentelle Pathologie und Pharmakologie, 1902, xlviii, p. 410.

[5] PAVY, BRODIE, and SIAU, in the Journal of physiology, 1903, xxix, p. 467, claim for PAVY the priority of this idea, referring to an article in the Lancet, 1902, p. 816. But the wording of the Lancet article gives a different impression. It reads as follows: "In both pancreatic and phlorhizin glycosuria there was a parallelism between sugar and nitrogen eliminations, showing that the sugar was derived from proteid catabolism, but various facts pointed to the sugar formation occurring in the latter case solely in the kidney, and in the former in the general system. It seemed probable that under the influence of phlorhizin the renal cells acquired the power of catabolizing proteid with the liberation of dextrose."

[6] LOEWI: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvii, p. 68.

tions in the ratio are not due to feeding fat, which accords with Lusk's [1] previous statements.

High ratios, after feeding very large quantities of fat, have indeed been obtained by Hartogh and Schumm,[2] but in this case there may have been a large fatty acid excretion in the fæces, and the balance of glycerin belonging to the fatty acid may have been synthesized to dextrose, for Cremer [3] has shown such a synthesis after feeding glycerin in phlorhizin diabetes.

As a result of this evidence, we can claim that the usual D : N ratio, in dogs living on proteid and fat, varies between 3.40 and 3.90 : 1, or a sugar production from proteid of between 62 and 54 per cent. In the following paragraph another cause which may produce a variability in the ratio will be shown.

The Protection from Combustion of Sugar Formed from Proteid in the Organism.

We have already made the statement that there is a total phlorhizin diabetes within certain limits, but these limits must not be overstepped by flooding the organism with sugar. This is true as regards proteid sugar as well as sugar directly fed. An example of this occurs in Loewi's [4] work. A dog was fed with 250 gms. of meat and received 2.5 gms. of phlorhizin every eight hours ; the average ratio was D : N : : 3.6 : 1. During the next 24 hours, 500 gms. meat and 5 gms. of phlorhizin were given every eight hours ; the ratio fell to 2.6 : 1. The following day the feeding was reduced to that first mentioned, and the ratio rose to 3.7. On the day 1500 gms. of meat were fed, 136 gms. of sugar were excreted ; whereas 161 gms. were called for, as indicated by the proteid metabolism. Probably a part was burned, since the kidney could not effect its removal with sufficient rapidity. Loewi suggests that more phlorhizin might have brought about a complete elimination of the sugar on the day when 1500 gms. of meat were fed. A part of Loewi's experiment, representing eight hour periods, is here reproduced (page 72).

Two comments upon this experiment occur to us. In the first

[1] Lusk : Zeitschrift für Biologie, 1901, xlii, p. 34.

[2] Hartogh und Schumm : Archiv für experimentelle Pathologie und Pharmakologie, 1900, xlv, p. 11.

[3] Cremer : Münchener medicinische Wochenschrift, 1902, p. 944.

[4] Loewi : Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvii, 47, p. 54.

place, the nitrogen of Period 1 on the third day is less than in Period 3 of the first day, although more meat was fed on the third day period, and the influence of the larger meat feeding of the second day must have been active eight hours after the last portion of 500 gms. of meat.[1] Perhaps some burning of proteid sugar took place, sparing some of the proteid from combustion.

The second comment is on the evenness of the ratios following the feeding of meat in excess. Reilly, Nolan, and Lusk found that if 500 gms. of meat were fed to a phlorhizinized dog (Dog V a), the sugar and nitrogen can be eliminated twelve hours after feeding.

LOEWI'S EXPERIMENT.

Period.	D.	N.	D : N.	Meat.
3	28.86	8.18	3.6	gms. 250
1	42.12	14.95	2.9	500
2	51.48	12.23	2.3	500
3	43.29	16.25	2.6	500
1	29.64	7.78	3.8	250
2	31.20	8.90	3.5	250
3	30.42	8.31	3.7	250

But on repeating this experiment four days later (Dog V b), there was a lag in the sugar excretion indicating decreased kidney function. In another dog a similar lag took place after feeding 870 gms. at one meal. But this sugar which was retained was not burned, but was eliminated in the after periods. This is shown in the table of ratios given on the following page.

Within limits, therefore, proteid sugar seems to be protected from combustion while awaiting removal by the kidney. The power of the phlorhizinized organism to retain proteid sugar unburned is also illustrated by the following experiment. In some of our work it was inconvenient to give phlorhizin later than 11 or 11.30 P. M., or earlier than 8.30 or 9 A. M. Consequently there was a night period of nine to ten hours during which it seemed possible that the phlorhizin effect

[1] Reilly, Nolan, and Lusk: *Loc. cit.*, p. 402.

might begin to wear off and the sugar be retained to some extent. The supposition was strikingly confirmed. In the table of our work on Dog II (p. 77), the preliminary period of the first day is given as fifteen hours. The urine of this period was collected in two portions.

REILLY, NOLAN, AND LUSK'S EXPERIMENTS.

	Dog V a. D : N.	Dog V b. D : N.	Dog VI. D : N.
Fasting, 12 hours	3.41	3.71	3.42
Meat, 12 hours	3.54	3.25	3.04
Following 12 hours . . .	3.56	3.95	3.25
Following 6 hours	....	4.12	....
Following 24 hours . . .	....	....	4.19
Total, subsequent to meat		3.53	3.45

The first represented the twelve hours from 9.10 P. M., June 10, to 9.10 A. M., June 11. The phlorhizin (2 gms.) was given at 11 P. M., and hence the last hour of this period was the tenth since the injection. The second portion of the urine was collected from 9.10 A. M. to 12.10 noon, three hours immediately following an injection of phlorhizin. The analyses showed the following:

	N.	D.	D : N.
I, 12 hours	8.74	29.18	3.33
II, 3 hours	2.28	10.04	4.40
Total	11.02	39.22	3.56

The interpretation is clear. During the latter part of the first period the sugar elimination lagged, and there was an accumulation in the system. Under the influence of a second dose of phlorhizin this accumulated sugar was swept out in addition to the dextrose properly belonging to the period. Consequently the sugar of the first period is too low and that of the second much too high. Com-

bining the two we have results for fifteen hours which there is every reason to regard as trustworthy.

Ten hours is then a longer time than should be allowed to intervene between injections, when it is desired to collect the urine for short periods. It will be noticed that the nitrogen excretion remained practically constant, instead of falling and rising with that of the sugar. We cannot suppose that the dextrose which was held back was burned to any appreciable extent; it seems to have appeared almost if not quite quantitatively in the second period.

At the close of this experiment (Dog II) we made another trial on the following plan. The usual dose of phlorhizin, 2 gms., was given at the beginning of a twelve hour period. This period was subdivided into periods of eleven hours and one hour, with the expectation that the urine of the last hour, the twelfth since the injection, would show a considerable lessening in sugar content. The findings follow:

Period.	N.	D.	D : N.
11 hours . . .	7.06	25.58	3 62
1 hour . . .	0.74	2.46	3.33

Phlorhizin was then given and the urine for three hours analyzed.

Period.	N.	D.	D : N.
3 hours . . .	1.86	7.21	3.87

An inspection of these figures shows that our expectations were not met. The dextrose in the twelfth hour after the first injection was equal to or slightly above the average for the fifteen hours. The fall of the ratio in this hour was due to a slight rise of the nitrogen above the mean. As to the reason why the phlorhizin effect was distinctly impaired on June 11, when *ten* hours had elapsed since the injection, while on June 13 it was not diminished in *twelve* hours, it can only be suggested that the longer time a dog has been receiving phlorhizin the more susceptible it becomes to its action, so that the effect of each successive dose tends to be more prolonged. The duration of the action of phlorhizin therefore varies even in the same dog.

It is clear from the foregoing evidence that the phlorhizinized dog has some power of retaining proteid sugar unburned in his organism if the kidney is not in a position to eliminate it. Some irregularities in the ratio may be explained by the lack of maximal phlorhizin action on the kidney, during the later hours after injection. The duration of this maximal activity varies with the individual.

The Protection from Combustion of Dextrose Administered Subcutaneously.

If the phlorhizinized organism has the power of retaining for a time a certain quantity of proteid dextrose without burning it, then the subcutaneous injection of small amounts of dextrose in a fasting diabetic dog should result in the quantitative protection of such dextrose and its elimination in the urine. Perhaps there is a limited quantity of some body which, combining with dextrose, renders it non-combustible, — a compound which can be split by the phlorhizinized kidney.

Two out of three dogs showed the quantitative elimination of dextrose subcutaneously administered. We quote the successful experiments. The unsuccessful result in one dog may have been due to imperfect kidneys.

The general plan of the method adopted was as follows. The animal was given phlorhizin subcutaneously (in 1.2 per cent sodium carbonate solution) three times daily until — by the third or fourth day — the diabetic condition was well established and the elimination of nitrogen and dextrose was proceeding evenly. After one or more preliminary periods, during which the urine was collected and analyzed, the subcutaneous injection of sugar was made. The dextrose used was a pure preparation (Kahlbaum's). A 0.5 per cent solution of this dextrose, made up by weight, gave a value of 0.515 per cent, as determined by the Allihn method.

After the injection the urine was collected for periods of three or six hours, and the amount of extra sugar eliminated, as compared with that before the injection, could be noted. Six hours after the introduction of the dextrose, the values had reached practically the initial level.

This experiment shows, (1) an unusually high ratio elsewhere commented upon. (2) The maintenance of this high ratio during a

twelve-hour period, when 150 gms. of meat had been fed and only 0.5 gms. of phlorhizin. The smaller dose of phlorhizin likewise

DOG I.

Fasted three days; then 3 gms. phlorhizin every eight hours for two days.
Weight = 18 kg.

Date. Mar.		Phlorhizin.	Urine.	D.	N.	D:N.	Extra sugar.	Remarks.
		gms.						
18	8.40 A.M.	5.0						
	11.40 A.M.	5.0	3.18–6.18 P.M.	8.89	2.188	4.06		
	9.30 P.M.	1.0						
19	8.45 A.M.	0.5	9.19–12.19	9.58	2.16	4.44	....	12.30–12.35 5 gms. D subcut. in 50 c.c. water.
	12.15 P.M.	0.5	12.19–3.19	13.87	2.10	....	4.64	
			3.19–6.19	9.85	1.96	....	1.22	
	9.45 P.M.	0.5	6.19 P.M. to	....	....	....	....	6.20 P.M. 150 gms. meat.
20			8.45 A.M.	49.79	11.47	4.34		

sufficed for the elimination of the sugar subcutaneously injected. (3) The apparent elimination of extra sugar to the amount of 5.86 gms. six hours after the subcutaneous injection of 5 gms. of dextrose. The extra sugar is obtained by multiplying the nitrogen for the period by the prevailing D : N ratio, which in this case is 4.39 (average of 4.44 and 4.34) and subtracting this proteid sugar from the total as determined in the urine.

A second experiment on similar lines ran the following course. (See Table, Dog II.)

This experiment is a type of the total phlorhizin diabetes. The average D : N ratio (excluding the sugar-injection periods) is 3.60 : 1. On June 11, after injecting 5 gms. of dextrose subcutaneously, extra sugar amounting to 5.47 gms. were found in the urine. On June 12, after injection of 4 gms., 3.43 gms. of extra sugar were eliminated through the kidney in six hours. The figures here and in the previous experiment demonstrate the quantitative elimination of dextrose administered in small quantities. We have seen that dextrose in excess is burned, but in small quantity it apparently enters into a chemical combination which is not combustible, and which may be split by the phlorhizinized kidney.

DOG II.

Fasted three days; then three days with 2 gms. phlorhizin every eight hours.
Weight = 18 kg.

Date. 1903.	Period.	D.	N.	D : N.	Extra D.	Remarks.
June 11	I, II, 15 hours	39.22	11 02	3.56		
	III, 6 hours	20.28	4.32	....	4.73	5 gms. dextrose in 50 c.c. water, subcutaneously.
	IV, 3 hours	8.01	2.05	....	0.74	
12	V, 12 hours[1]	28.78	7.99	3.60		
	VI, 3 hours	7.53	2.06	3.65		
	VII, 6 hours	17.90	4.02	....	3.43	4 gms. dextrose, subcutaneously.
	VIII, 3 hours	7.07	1.93	3.66		
13	IX, 11 hours	25.58	7.06	3.62		

[1] 5 gms. phlorhizin at 11 P. M.

This experiment has been used before in this article. It will be noticed that Periods I and II are added together, because the evening injection of phlorhizin did not cause the complete elimination of

HOURLY EXCRETION, DOG II.

Period.	D. per hour.	N. per hour.	D : N.
I, II, 15 hours . . .	2.61	0.735	3.56
III, 6 hours	....	0.720	
IV, 3 hours	....	0.683	
V, 12 hours	2.39	0.666	3.60
VI, 3 hours	2.51	0.687	3.65
VII, 6 hours	....	0.670	
VIII, 3 hours . . .	2.36	0.643	3.66
IX, 11 hours	2.32	0.642	3.62

all the sugar, during the night, but this all came out in the three hours following the morning injection. Five gms. at 11 P. M. on June 12 caused a maintenance of the ratio, and during the night of

June 13 2 gms. sufficed for eleven hours — something not possible two days before. Arteaga[1] found that 0.1 gm. doses become increasingly effective in the cat. It has been a recent custom in our laboratory to give 5 gm. doses for a day or two, and then 2 gms. of phlorhizin. The ratio is then usually established on the second day.

The evenness of the elimination of nitrogen and dextrose is remarkable. In the experiment on Dog II the hourly excretion of sugar and nitrogen is presented in a table on p. 77. The nitrogen and dextrose constantly and slowly fall together, the ratio remaining the same.

It must be noted in such experiments as these that after withdrawing the urine by a catheter, the bladder should be washed three times with warm water, and this operation should be finished and the catheter quickly withdrawn within half a minute of the appointed time. This is easily accomplished.

Conclusion.

It may be of advantage to state concisely what this discussion indicates concerning the nature of phlorhizin diabetes. If phlorhizin be administered in 2 gm. doses every eight hours to fasting dogs, there is a preliminary sweeping out of the body's sugar, and thereafter there is established a ratio between nitrogen and dextrose in the urine of 3.75 : 1 (or 3.60 to 3.70, perhaps, in the light of more numerous experiments than were at first at hand). If the phlorhizin action on the kidney be complete, this ratio remains constant even in three-hour periods. After feeding meat, gelatin, or casein, the ratio is unchanged in the aggregate, although the sugar tends to be eliminated before the nitrogen belonging to the proteid fed.[2]

Dextrose fed in small quantities *per os* or injected subcutaneously is not burned, but eliminated quantitatively. Diminished action of phlorhizin may lower the ratio for a time, but quick renewal of the phlorhizin will bring about a ratio higher than normal, on account of the fact that sugar from proteid has accumulated within the body without being burned. The aggregate of two such periods shows the normal ratio. Phlorhizin diabetes is therefore a total diabetes. Dextrose within limits cannot be burned. It seems possible to ac-

[1] Arteaga: *Loc. cit.*

[2] Reilly, Nolan, and Lusk: *Loc. cit.*

cept Loewi's hypothesis of a blood-sugar combination, with the additional hypothesis that the sugar while in this combination cannot be burned. Phlorhizin will decompose it and permit the elimination of the sugar in the kidney. Any free dextrose unites with the combining radical and is protected. If the quantity of sugar rises above this combining power, immunity from destruction is lost and the sugar burns.

Vosburg and Richards [1] have shown that the blood-sugar increases in that form of diabetes which is produced by the effect of intraperitoneal injections of adrenalin upon the pancreas. After such injection, diabetes usually ensues within a few minutes. In one experiment by Herter and Richards,[2] a fasting dog was treated with phlorhizin and the urine tested until it was sugar-free. Intraperitoneal injection of adrenalin failed to produce glycosuria within four hours, but during the following four hours a small amount of sugar was eliminated. This can be explained if this dog's organism, freed from dextrose through phlorhizin, possessed at first a sugar-combining power (in Loewi's sense, with colloid) which, when satisfied, was followed by that extra accumulation of free blood-sugar which Loewi claims to be necessary for sugar elimination through the kidney.

If phlorhizin diabetes is to be produced, animals with sound kidneys are essential. Our experience has taught us never to use the same animal at different dates, for the first experiment may have done violence to the kidneys and the 2.8 ratio is likely to be obtained. In this case the kidney has lost the power of splitting a dextrose combination formed from a definite percentage of the proteid sugar, a compound which is always burned in animals having the lower ratio.

[1] VOSBURG and RICHARDS: This journal: 1903, ix, p. 35.

[2] HERTER and RICHARDS: Medical news, 1902, lxxx, p. 201.

EXPERIMENTS ON THE DIGESTIBILITY OF VEGETABLES.

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CONTENTS.

Introduction.

THE experiments here reported were carried on during the summer of 1901, but the analytical work was not completed until early winter, and the preparation of this report has been unavoidably delayed until the present time (July, 1903). The authors are indebted to Professor W. O. Atwater, Chief of Nutrition Investigations of the United States Department of Agriculture, for material assistance in making the analyses and determinations of the heats of combustion of the food materials and excretory products; also to Mr. H. A. Pratt for help in carrying out the details of the experiments, and to Mr. F. E. Woodworth who served as one of the subjects.

Previous Work.

Regarding the digestibility of vegetables by man, that is, the proportions of their nutrients that are digested and absorbed during their passage through the alimentary tract, the amount of information hitherto available has been small, and has come mostly from early work of some European investigators. Rubner[1] has reported one experiment with each of the following: potatoes, carrots, cabbage, green beans, and dried peas. Constantinidi[2] made two experiments with potatoes, Strumpell,[3] one with dried lentils, and Prausnitz,[4] one with dried beans. The data from these few experiments have formed practically the basis of the usual estimates of the digestibility of the nutrients of this class of food materials. More recently, some attention has been paid to the subject in this country also: Snyder[5] has studied the digestibility of potatoes and of beans, and an extended investigation with legumes is being prosecuted by Wait[6] at the present time. The digestibility of fruits and nuts is being studied by Jaffa.[7] With the exception of a few experiments by Japanese investigators, the reports of which were not accessible to the present writers, these are the only complete studies by modern methods that were found on record.

Studies of the digestion of food in the stomach (chymification), such as the classical observations of Beaumont[8] and those of a similar nature by later investigators, are not considered here, as these have to do rather with the ease or time of digestion than with the amounts digested; and the results of artificial digestion experiments, which

[1] Rubner: Zeitschrift für Biologie, 1879, xv, pp. 147, 166, 168; 1880, xvi, pp. 127, 128.

[2] Constantinidi: Zeitschrift für Biologie, 1887, xxiii, p. 447.

[3] Strumpell: Konig's Chemie der menschlichen Nahrungs- und Genussmittel. Third edition, p. 46.

[4] Prausnitz: Zeitschrift für Biologie, 1890, xxvi, p. 227.

[5] Snyder: United States Department of Agriculture. Office of Experiment Stations, Bulletin No. 43, p. 21, and Minnesota Experiment Station, Bulletin No. 74, p. 121.

[6] Wait: Not yet published.

[7] Jaffa: United States Department of Agriculture. Office of Experiment Stations, Bulletin No. 107, Bulletin No. 132.

[8] Beaumont: The physiology of digestion, with experiments on the gastric juice. Second edition.

are sometimes interpreted as indicating the proportions of materials that may be digested, are likewise neglected because the conditions of the experiments differ too much from those of digestion in the body.

Experiments Here Reported.

The object of the experiments here reported was to study the digestibility of representatives of some of the more important classes of vegetables, but the results also throw some light on the effect of the different vegetables upon the digestibility of other materials in the diet of which they form a part. The potato was taken as representative of the most important class of vegetables in a large section of the country; the cabbage was selected as typical of succulent vegetables, and the beet as a type of roots. Apple sauce was also studied, because it is not infrequently used as a substitute for some vegetable, and because apples are one of the cheaper and commoner fruits; and one experiment was made with green corn, to learn whether it is as indigestible as is commonly supposed.

The subjects in these experiments were three young, healthy men, with good appetites and normal digestive functions. Two of them were the authors themselves, and the third was a college student. Subject B. was thirty-three years old, five feet eleven inches in height, and weighed without clothing about sixty-six kilograms (145 lbs.). His regular work was more or less sedentary in character, but he was of active disposition, and in addition to his work, which continued from 8 A.M. to 4.30 P.M. each day, he spent considerable time in more active out-door exercise. Subject M. was thirty-one years old, five feet ten inches in height, and weighed without clothing about seventy-four kilograms (163 lbs.). As was the case with the former subject, his occupation was sedentary, but he took considerable active exercise each day before and after work. Subject W. was twenty-eight years old, a little over five feet in height, and without clothing weighed about fifty-three kilograms (117 lbs.). Throughout the time of the experiments he was engaged ten hours each day at hard out-door work.

Each of the materials enumerated above was eaten separately in an experiment of three days' duration. The green corn, however, was eaten by but one of the subjects, and the apple sauce by two; but with the other three materials parallel experiments were made with all three of the subjects.

Experimental methods. — In these investigations it was necessary for us to devise a method of procedure. In the European experiments previously referred to the different materials were eaten almost alone, but there are several objections to such a course. In the first place, it is difficult for the subject to continue the experiment long enough, for the monotonous diet soon becomes distasteful, and commonly the digestive processes are disturbed to such an extent that the results are impaired. On the other hand, even if the diet could be endured, the results thus obtained would be of doubtful value. The digestibility of a single food material as thus determined might not be the same as that of the same material when combined with others in a mixed diet; for the digestion of one material may be increased or diminished by the ingestion of others with it.

Experiments with men on a mixed diet may be continued for a considerable period, even when the number of materials is small, and the digestibility of a given material in such a diet may be satisfactorily calculated, when that of the foods with which it is eaten may be estimated for the conditions of the experiment. Woods[1] and Snyder[2] have in this way estimated the digestibility of bread from the results of experiments with bread and milk. The ingredients of the fæces that would be derived from the milk they calculated by use of previously determined factors for digestibility of milk, and they assumed that the remaining ingredients of the fæces would be due to the bread.

No such simple diet, however, was possible in these experiments with vegetables. It was, therefore, necessary to devise a plan by which the digestibility of the vegetables alone could be estimated from the results obtained with a diet of several materials, including those under observation. The method adopted was as follows: A diet of meat, bread, butter, milk, and sugar was chosen, the same number and kind of materials for each subject, and this was eaten for several meals, until each one had found what quantities would be most agreeable to him. The diet thus decided upon was termed the basal ration, and an experiment of three days was then made with each subject, to determine the digestibility of the ration he had chosen. The results thus obtained were employed as factors, as explained below.

[1] Woods: United States Department of Agriculture. Office of Experiment Stations, Bulletin No. 85, p. 16.

[2] Snyder: United States Department of Agriculture. Office of Experiment Stations, Bulletin No. 101, p. 21.

It was intended that the proportions of the different materials in the basal ration decided upon by each subject should be kept the same from day to day and from experiment to experiment. A subject might increase or decrease the total quantity, according to his bodily needs or the dictates of his appetite, but the quantity of each material was to be increased or decreased so that the proportion of one to another was to be the same whether the ration was large or small. With one subject the ration decided upon was maintained uniformly from meal to meal throughout all the experiments, which were consecutive without intermission. The other subjects found it necessary to make slight variations, and in a few cases the proportions of the different materials were by mischance slightly changed; but a comparison of the results in these cases with these two subjects, and those in the same experiments with the other subject, showed that the alterations were not sufficient to affect the digestibility of the diet to any appreciable extent.

To the basal ration the different vegetables were added singly, in as large quantities as possible, in successive three day periods, and the digestibility of the total diet, including the vegetable, was determined. By means of the factors for the digestibility of the basal ration previously ascertained, estimates were made of the quantities of ingredients in the fæces that should be due to this part of the diet, and the remaining ingredients in the fæces were assumed to pertain to the vegetable alone; and the difference between these and the nutrients in the vegetable eaten was taken as a measure of the digestibility of the latter.

In these experiments the digestibility of the nutrients was determined in the usual manner, by collecting the fæces pertaining to a given diet, analyzing samples of the food materials and fæces in the same way, and deducting from the quantities of nutrients in the food consumed the amounts of the corresponding ingredients in the fæces. It is, of course, understood that this method involves several errors, but under the present circumstances these are unavoidable. Perhaps the most serious error is in the analysis of the fæces. These are made up of not only the undigested portions of food, but also the so-called metabolic products, which consist of unabsorbed residues of digestive secretions, fragments of epithelium from the alimentary canal, and various excretory materials; and among which are certain substances that, according to the methods of analysis followed, would be determined as ingredients of undigested food. For

instance, the nitrogen in the digestive secretions not re-absorbed would be included with that of undigested protein; while ether extract of the fæces considered as undigested fat would include other substances, such as bile products, etc., that are also soluble in ether. But as no satisfactory method has yet been devised for separating the undigested residues of food from the metabolic products in the fæces, it is customary to consider the total fæces as if composed of undigested food. The effect of the error involved in this method, however, is to make the values for digestibility too small; that is, the results are within rather than beyond the truth.

Treatment of the fæces. — In digestion experiments of this kind the accuracy of the results depends largely upon a satisfactory separation of the fæces for a given diet from those pertaining to food eaten before or after the experimental period. In these investigations an experiment with one diet began with breakfast, immediately following the supper of the last day of the preceding experiment with another diet. Separation of the fæces for the different diets was accomplished by means of lampblack, taken in gelatin capsules, either just after the last meal of one experiment, or just before the first meal of the next one, the coloration of the fæces by the lampblack, and the difference in their character due to changes in the vegetables, generally affording means for satisfactory determination of the point of separation.

The fæces for each experiment were deposited in tared dishes, and dried in a water-jacketed oven below the temperature of 100° C. When dried, they were covered loosely and allowed to stand for twenty-four hours, after which they were again weighed, and the total amount of fæces for each experiment finely ground for analysis.

Sampling of food materials. — In order to avoid much of the large amount of labor involved in separate analyses of the food materials forming the basal ration in each experiment, and also to obviate the necessity of the subjects eating at a common table, the following plan of sampling the ingredients of the basal ration was adopted: One-twentieth of the weight of each of the food materials eaten was taken as a sample, and all were placed together to form a composite sample of the total basal ration. Subjects B. and W. took these samples at the close of each day, while M. took samples at each meal. The samples were placed in jars having wide mouths, in order that they might be removed completely, and a few drops of formaldehyde were added in each jar as a preservative. These composites were prepared

with much care, for a mistake is weighing or sampling one material would destroy the accuracy of the sample for the whole ration.

The sample of each material was made as nearly as possible representative of that eaten; thus, with bread, where the crust and crumb were eaten, as in experiments with B. and W., especial care was taken that the proportion of crust to crumb should be the same as in the bread consumed. With Subject M., the crust was not eaten, hence only the crumb was sampled. The meat, butter, and sugar were of uniform composition throughout each experiment with all subjects, and presented no difficulty in sampling, the meat being taken from a large lot specially prepared for experimental work, and containing very little water, fat, or extractives. The milk was thoroughly shaken each time before using, to insure a uniform distribution of the fat, and the same was done before the sample was taken.

Samples of the vegetables were taken separately, without regard to the amount eaten.

At the close of each study the composite sample of each basal ration was completely removed from the jar, dried in a water oven, and prepared for analysis in the usual manner.

The analyses of the basal ration, the vegetables and the fæces for the different experiments, which were made according to the methods of the Association of Official Agricultural Chemists,[1] and the heats of combustion, which were made by means of the bomb calorimeter,[2] are shown in Table I.

Details of the experiments. — With two subjects, B. and W., similar studies were made simultaneously, while those with W. were at very near the same time, each subject having his meals prepared and eating them at home, scales being at hand for the weighing of the materials as eaten.

Experiments with Subject B. — Of the six experiments with this subject the first was with a large basal ration, and the second was with an ordinary basal ration, without any vegetable; after which followed, in the order named, studies of cabbage, potatoes, beets, and apple sauce. Each experiment continued three days and was followed immediately by the next one, the lampblack for the separation of the fæces being taken at the beginning of the first meal of each study. The weight of the subject, which was taken each day, varied but little during the series.

[1] United States Department of Agriculture. Bureau of Chemistry, Bulletin No. 46.

[2] Storrs: Experiment Station Report, 1897, p. 199.

TABLE I.

COMPOSITION OF FOOD MATERIALS AND FÆCES.

Sample No.	Material.	Experiment No.	Water.	Protein.	Fat.	Carbohydrates.	Fibre.	Ash.	Heat of combustion per gram.
			per cent.	p.cent.	p.cent.	p.cent.	p.cent.	p.cent.	calories.
3366	Basal ration composite	1	74.2	3.9	6.1	14.9	0.2	0.9	1388
3367	" " "	2	73.4	4.4	5.8	15.5	..	0.9	1409
3368	" " "	3	72.5	4.2	5.6	16.8	..	0.9	1392
3370	" " "	4	74.3	4.6	5.8	14.3	..	1.0	1368
3372	" " "	5	75.0	4.1	5.5	14.5	..	0.9	1311
3374	" " "	6	75.5	4.2	5.5	13.9	..	0.9	1305
3376	" " "	7	63.0	6.3	8.0	21.4	0.2	1.3	1980
3377	" " "	8	64.2	5.8	7.6	21.3	..	1.1	1890
3379	" " "	9	62.6	6.4	7.0	22.9	..	1.1	1951
3381	" " "	10	59.4	7.1	9.0	23.3	..	1.2	2176
3383	" " "	11	67.1	7.1	6.2	18.7	0.2	0.9	1745
3385	" " "	12	67.8	6.7	6.4	18.2	..	0.9	1649
3387	" " "	13	66.5	6.7	7.7	17.9	..	1.1	1770
3389	" " "	14	66.8	6.6	6.4	19.1	..	1.1	1776
3391	" " "	15	70.9	6.2	5.4	16.5	..	1.0	1545
3392	" " "	16	69.1	6.5	5.9	17.6	..	0.9	1652
3369	Cabbage	3	94.7	0.9	0.3	3.3	1.1	0.8	214
3378	"	8	94.8	1.0	0.3	3.0	..	0.9	210
3386	"	12	94.4	0.9	0.1	3.7	..	0.9	203
3371	Potatoes	4	79.5	2.2	0.1	17.4	0.4	0.8	848
3380	"	9	78.3	2.3	0.1	18.4	..	0.9	900
3384	"	11	81.2	1.9	0.3	15.5	..	1.1	782
3373	Beets	5	82.6	1.9	0.3	13.8	1.0	1.4	673
3382	"	10 & 13	85.4	2.2	0.2	10.8	..	1.4	599
3375	Apple sauce	6	77.6	0.3	0.6	21.3	0.9	0.2	875
3390	" "	14	78.8	0.2	0.1	20.7	..	0.2	838

TABLE I — (*continued*).

Sample No.	Material.	Experiment No.	Water.	Protein.	Fat.	Carbohydrates.	Fibre.	Ash.	Heat of combustion per gram.
			per cent.	p.cent.	p.cent.	p.cent.	p.cent.	p.cent.	calories.
3393	Green corn	16	76.0	4.9	1.4	17.3	0.5	0.4	1112
3394	Fæces (fresh)	1	77.4	7.5	3.7	7.3	0.2	4.1	1346
3395	" "	2	79.7	7.6	3.9	4.4	0.2	4.4	1124
3396	" "	3	86.7	4.3	2.2	4.0	1.4	2.8	676
3397	" "	4	86.8	4.5	3.4	2.3	0.8	3.0	632
3398	" "	5	79.1	7.7	2.8	6.0	1.0	4.4	1050
3399	" "	6	81.2	7.5	3.0	4.6	0.6	3.7	980
3400	Fæces (partially dried)	7	8.7	31.1	22.5	17.0	0.9	20.7	5278
3401	" " "	8	10.0	30.0	15.7	25.1	6.0	19.2	4728
3402	" " "	9	7.9	38.3	16.3	18.5	2.5	19.0	4750
3403	" " "	10	5.0	34.2	24.7	15.4	3.5	20.7	5118
3404	Fæces (fresh)	11	77.2	10.1	4.9	3.4	0.5	4.4	1255
3405	" "	12	79.8	8.0	3.4	4.7	0.8	4.1	1050
3406	" "	13	85.1	5.9	2.4	3.2	0.8	3.4	739
3407	" "	14	72.3	12.5	4.6	5.6	0.9	5.0	1462
3408	" "	15	72.0	10.8	7.0	3.8	0.3	6.4	1482
3409	" "	16	82.8	5.9	4.9	3.1	0.7	3.3	937

Experiments with Subject M. — The studies with this subject comprised one experiment with a basal ration alone, and one each with cabbage, potatoes, and beets added. An attempt was made to carry on an experiment with a large basal ration, while a similar one was being made with B., but on the last day there was a diarrhœa which interfered with the test. The first three experiments were three days each, but the fourth had to be discontinued after the second meal of the second day. The body-weight of the subject, which was taken only twice, was 74.15 kilograms at the beginning of the first study and 73.70 kilograms after the close of the last one. This subject was unable to consume the basal ration with such exact uniformity as was done by the preceding subject, and the proportions of the

different materials were altered a trifle in the first two studies; but considering the total amounts for the periods, the discrepancies were too small to influence the results. The weights of the fæces for the experiments with this subject are given in Table II, for the partially dried material, as the fresh fæces were not weighed.

Experiments with Subject W. — Six experiments were made with this subject in the following order: potatoes, cabbage, beets, apple sauce, basal ration, and green corn. For several days before entering the experiments, the subject had been living on the diet that he adopted as the basal ration, and an attempt was made to determine the digestibility of the diet during the last three preliminary days; but, owing to a poor separation of the fæces, this was unsatisfactory. Inasmuch as provision had already been made for the experiments with vegetables, the test of the basal ration was not repeated until later.

The basal ration in this series consisted of the same materials as in experiments with the other subjects. The total quantities eaten were varied by the subject on different days, according to his appetite; but with one unimportant exception, the relative proportions of the different materials were maintained uniform; that is, each one was increased or diminished a certain fraction. In calculating the digestibility of the vegetables in such cases, it was necessary to assume that such variations would not affect the digestibility of the basal ration; but the error introduced is probably insignificant.

The first experiment in this series continued four days, the others three days each; and as was the case with the other subjects, one experiment followed another without interruption, the lampblack being taken at the beginning of the first meal in each study. The subject generally weighed himself without clothing at the beginning and end of each experiment.

Results of the experiments. — The results of the experiments are given in Table II, showing for each the digestibility of the nutrients of the total ration, including the vegetable, determined as before explained, and the estimated digestibility of the nutrients of the vegetables alone. The manner of making these estimates may be briefly illustrated by use of figures from Experiment No. 3. In this case the basal ration furnished 171 grams of protein, of which, according to the results of Experiment No. 2, there would be 89 per cent digested, while 11 per cent, or 18.8 grams, would appear in the fæces, leaving 13.5 grams of the protein of the fæces as coming from the cabbage.

Since the total amount of cabbage consumed contained 19.6 grams of protein, the above computation would indicate that 6.1 grams was digested, or 31.1 per cent of the whole.

It is obvious that such computation is based on the assumption that the digestibility of the basal ration was the same when the vegetables were added as when they were not. While undoubtedly this is not strictly true, nevertheless the estimates thus made are probably closer approximations to the truth than could be obtained by use of separate factors for the digestibility of the different materials of the basal ration.

It will be observed that the estimates of the digestibility of protein, and especially of fat, vary widely for the different subjects in several instances. This may be due to several causes, but the chief one is undoubtedly the fact that when the proportion of the nutrient in the vegetable was particularly small, as compared with that in the basal ration, a small error in the assumed factor for the digestibility of the given nutrient in the basal ration would have a large effect upon the estimate for that in the vegetable. The results for the carbohydrates of the vegetables, the proportions of which in the diet were relatively quite large, were, in all cases but one, uniform and satisfactory, the exception being the cabbage, in which the proportion of carbohydrates to the total amount in the diet was smaller than for any other vegetable. So far as the vegetables used in these experiments are concerned, the results for carbohydrates are of most importance, as no one of them is of much significance as a source of either protein or fat.

Although the number of studies is too small to warrant final conclusions, some deductions from the results obtained in the experiments are worthy of consideration.

Effect of size of the ration. — Preceding investigators[1] have found that small rations were somewhat more thoroughly digested than larger ones of the same kind. In the series of experiments with Subject B. the size of basal ration appeared to make but little difference in the proportions digested. The results indicated a slightly larger digestibility for the smaller ration, though no more than might be expected unless the effect of the metabolic products were to keep the quantity of fæces about the same in all cases.

Variations in individuals. — In some cases there were quite marked differences in the extent to which the food eaten was utilized by the

[1] See SNYDER: *Loc. cit.*

TABLE II.

Data of the Experiments.

Sample No.		Total weight.	Protein.	Fat.	Carbo-hydrates.	Fibre.	Ash.	Energy.
	Experiment No. 1.	grams.	grams.	grams.	grams.	gms.	gms.	cal.
3366	Basal ration	6105.0	238.1	372.4	909.7	12.2	54.9	8474
3394	Fæces	404.5	30.3	15.0	29.5	0.8	16.6	544
	Digestibility of total ration, % . .	....	87.3	96.0	96.8	93.4	69.8	93.6
	Experiment No. 2.							
3367	Basal ration	4071.0	179.1	236.1	631.0	8.1	36.7	5736
3395	Fæces	286.9	21.8	11.2	12.6	1.9	12.6	322
	Digestibility of total ration, % . .	....	89.0	95.3	98.0	92.6	65.4	94.4
	Experiment No. 3.							
3368	Basal ration	4071.0	171.0	228.0	683.8	8.1	36.6	5666
3369	Cabbage	2180.0	19.6	6.5	71.9	24.0	17.5	467
3396	Fæces	752.2	32.3	16.5	30.1	10.5	21.1	508
	Digestibility of total ration, % . .	....	83.1	93.0	96.0	67.3	61.0	91.7
	Estimated digestibility of cabbage, %	....	31.1	10.8	77.2	58.7	52.0	59.1
	Experiment No. 4.							
3370	Basal ration	4071.0	187.2	236.1	582.2	8.1	40.7	5569
3371	Potatoes	2036.0	44.8	2.0	354.2	8.1	16.3	1726
3397	Fæces	523.1	23.5	17.8	12.0	4.2	15.7	331
	Digestibility of total ration, % . .	....	89.9	92.5	98.7	74.1	72.5	95.5
	Estim. digestibility of potatoes, % .	....	93.5	....	99.9	55.6	90.2	98.9
	Experiment No. 5.							
3372	Basal ration	4071.0	166.9	223.9	590.2	8.1	36.7	5337
3373	Beets	1987.0	37.7	6.0	274.2	19.9	27.8	1337
3398	Fæces	363.1	27.9	10.2	21.8	3.6	16.0	381
	Digestibility of total ration, % . .	....	26.4	95.6	97.5	87.1	75.2	94.3
	Estimated digestibility of beets, % .	....	74.8	....	95.7	84.9	84.5	93.9

TABLE II — (*continued*).

Sample No.		Total weight.	Protein.	Fat.	Carbo-hydrates.	Fibre.	Ash.	Energy.
	Experiment No. 6.		grams.	grams.	grams.	gms.	gms.	cal.
3374	Basal ration	4071.0	171.0	223.9	565.8	8.1	36.7	5313
3375	Apple sauce	3550.0	10.7	21.3	756.1	31.9	7.0	3106
3399	Fæces	380.5	28.5	11.4	17.5	2.3	29.6	373
	Digestibility of total ration, % . .	....	84.3	95.4	98.7	94.3	67.7	95.6
	Estim. digestibility of apple sauce, %	....	9.9	95.8	99.2	94.7	80.0	97.6
	Experiment No. 7.							
3376	Basal ration	4175.0	263.0	334.0	893.4	8.4	54.3	8266
3400	Fæces	38.7	12.0	8.7	6.6	0.3	8.0	204
	Digestibility of total ration, % . .	....	95.4	97.4	99.2	96.4	85.3	97.5
	Experiment No. 8.							
3377	Basal ration	3995.0	231.7	303.6	850.9	8.0	44.0	7550
3378	Cabbage	2315.0	23.2	6.9	69.4	25.5	20.8	486
3401	Fæces	63.8	19.1	10.0	16.0	3.8	12 3	302
	Digestibility of total ration, % . .	....	92.5	96.8	98.3	88.7	81.0	96.2
	Estimated digestibility of cabbage, %	....	63.8	69.6	86.7	86 3	67.3	76.5
	Experiment No. 9.							
3379	Basal ration	4080.0	261.1	285.6	934.3	8 2	44.9	7960
3380	Potatoes	1855.0	42.7	1.9	341 3	7.4	16.7	1670
3402	Fæces	55.4	21.2	9.0	10.3	1.4	10.5	263
	Digestibility of total ration, % . .	....	93.0	96.2	99.2	91.0	82.9	97.3
	Estim. digestibility of potatoes, % .	....	78.5	15.8	99.2	85.1	76 6	96 2
	Experiment No. 10.							
3381	Basal ration	2210.0	156 9	198.9	514 9	4.4	26.5	4809
3382	Beets	585.0	12.8	1.2	63.2	5.9	8.2	350
3403	Fæces	37.6	12 8	9.3	5.8	1.3	7 8	195
	Digestibility of total ration, % . .	....	92.5	95.4	99.0	87.4	78.4	96.0
	Estimated digestibility of beets, % .	....	56 2	....	97.3	81.4	52.4	78.6

TABLE II — (*continued*).

Sample No.		Total weight.	Protein.	Fat.	Carbo-hydrates.	Fibre.	Ash.	Energy.
	Experiment No. 11.	grams.	grams.	grams.	grams.	gms.	gms.	cal.
3383	Basal ration	4340.0	308.1	269.1	811.6	8.7	39.0	7573
3384	Potatoes	2402.0	45.6	7.2	372.3	9.6	26.4	1878
3404	Fæces	457.7	46.2	22.4	15.6	2.3	20.1	574
	Digestibility of total ration, % . .	....	86.9	91.9	98.7	87.4	69.3	93.9
	Estim. digestibility of potatoes, % .	....	47.4	....	98.0	82.3	62.9	85.6
	Experiment No. 12.							
3385	Basal ration	3851.0	258.0	246.5	700 8	7.7	34.6	6350
3386	Cabbage	2264.0	20.4	2.3	83.7	24.9	20.4	460
3405	Fæces	492.0	39.4	16.7	23.1	3.9	20.2	517
	Digestibility of total ration, % . .	....	85.8	93.3	97.0	88.0	63.3	92.4
	Estimated digestibility of cabbage, %	....	....	....	80.8	86.8	46.1	42.8
	Experiment No. 13.							
3387	Basal ration	4966.0	332.7	387.4	888.8	9.9	54.6	8790
3382	Beets	2884.0	68.4	5.8	311.5	28.8	40.4	1728
3406	Fæces	554.6	32.7	13.3	17.7	4.4	18.9	410
	Digestibility of total ration, % . .	....	93.4	96.6	98.5	88.6	80.1	96.1
	Estimated digestibility of beets, % .	....	86.3	100.0	97.2	86.8	89.1	96.6
	Experiment No. 14.							
3389	Basal ration	4125.0	272.2	264.0	788.0	8.3	45.3	7285
3390	Apple sauce	3330.0	6.7	3 3	689.0	30.0	6.7	2790
3407	Fæces	195.5	24.4	9.0	11.0	1.8	9.8	286
	Digestibility of total ration, % . .	....	91.3	96.6	99.3	95.3	81.2	97.2
	Estim. digestibility of apple sauce, %	....	28.4	100.0	99.6	95.7	100.0	100.0
	Experiment No. 15.							
3391	Basal ration	5504.0	341.2	297.2	908.1	11.0	55.0	8503
3408	Fæces	228.3	24.6	16.0	8.7	0.7	14.6	338
	Digestibility of total ration, % . .	....	92.8	94.6	99.0	93.6	73.5	96.0

TABLE II — (*concluded*).

Sample No.		Total weight.	Protein.	Fat.	Carbo-hydrates.	Fibre.	Ash.	Energy.
	Experiment No. 16.	grams.	grams.	grams.	grams.	gms.	gms.	cal.
3392	Basal ration	4255.0	276.6	251.0	748.9	8.5	38.3	7030
3393	Green corn	1738.0	85.2	24.3	300 7	8.6	6.9	1933
3409	Fæces	569.6	33.6	27.9	17.7	4.0	18.8	534
	Digestibility of total ration, % . .	....	90.7	89.9	98.3	76.6	49.4	94.0
	Estimated digestibility of corn, % .	....	83.9	41.2	96.6	59.3	..	86 2

three subjects. Subject B. appeared to get the least, and Subject M. the most, from the material consumed, though later in the series the latter subject was unable to continue with the diet, and there was a distinct decrease in the efficiency of digestion, as seen in the results of Experiment No. 10. In all cases, however, the differences in the proportions of carbohydrates digested by the different subjects were small; in fact, the uniformity was unusually satisfactory.

The digestibility of cabbage. — Of the vegetables studied in these experiments the results with cabbage were lowest. According to the estimates for the vegetable alone, the digestibility of protein was particularly low. Considering that cabbage contains so little protein, and that at least a half of it is non-proteid, the results for protein in cabbage are of little importance. The ether extract of cabbage, designated fat, probably consists of chlorophyll and other matters soluble in ether with little or no food value. On the average for the three subjects, 82 per cent of the carbohydrates was digested and utilized by the body. Of the total energy of the cabbage only 60 per cent was contained in the digested material, and when allowance is made for the energy of the incompletely oxidized portion excreted in the urine, not much over 57 per cent of the energy of the cabbage was actually available to the body.

In the experiment reported by Rubner, in which cabbage was the only article of the diet, the proportions digested were: protein, 81.5 per cent; ether extract, 93.9 per cent; carbohydrates, 84.6 per cent; and mineral matters, 80.7 per cent.

The digestibility of potatoes. — Considering the average of the three experiments with potatoes, about 73 per cent of the protein and 99 per cent of the carbohydrates appeared to be digested, while the energy of the digested material was 94 per cent of the total amount in the potatoes consumed. Correction for the energy lost in the unoxidized protein would indicate that about 91 per cent of the total energy of the potatoes was available to the body.

In Rubner's experiment, in which potato was eaten with butter, the digestibility of the nutrients was: for protein, 67.8 per cent; for ether extract, 96. 3 per cent; for carbohydrates, 92.4 per cent; and for mineral matter, 84.2 per cent. In one experiment by Constantinidi, in which suet and gluten were eaten with potatoes, the digestibility of the diet was: protein, 93.6 per cent; ether extract, 97.5 per cent; carbohydrates, 99.6 per cent; and mineral matters, 83.5 per cent. In a parallel experiment with the gluten omitted from the diet, the factors for the different nutrients were: protein, 80.5 per cent; ether extract, 98.8 per cent; carbohydrates, 99.3 per cent; and mineral matters, 87.7 per cent.

The digestibility of beets. — Although the beets were not particularly relished by either of the subjects, their digestibility was high in the experiments with all three, the average being: for protein, 72 per cent, and for carbohydrates, 97 per cent; while 90 per cent of the total energy of the beets was contained in the digested material. With allowance for the energy of incompletely oxidized material, at least 87 per cent of the total energy of the beets was actually available to the body. If the experiment with Subject M. were disregarded, because of the indisposition of the subject, the results would be still higher.

There is no other experiment with beets with which to compare the results from these. In Rubner's experiment with carrots, which closely resemble beets in composition, the factors for digestibility were: protein, 61 per cent; ether extract, 93.6 per cent; carbohydrates, 81.8 per cent; and mineral matters, 66.2 per cent.

The digestibility of apple sauce. — The factors for the digestibility of protein in the apple sauce were low for both subjects; but the quantity of protein present was too small to be considered. Some fat, however, was present, as the sauce was made of baked apples to which butter had been added, and the digestibility of the fat, as estimated for the apple sauce alone, averaged 98 per cent for the two experiments. The high factor for carbohydrates, averaging 99 per cent for

the two subjects, was no doubt favorably influenced by the sugar added in making the apple sauce. The digestible nutrients contained 99 per cent of the total energy of the apple sauce, nearly all of which was actually available to the body, because so little of it was derived from protein. In thirty experiments with fruits and nuts in various combinations Jaffa found the digestibility to be on the average: protein, 75, fat, 86, carbohydrates, 95, and crude fibre, 74 per cent.

The digestibility of fibre. — On the whole, the results for the digestibility of fibre were rather higher than might have been expected, though the materials were all eaten before they had fully ripened, and consequently the cellulose may have been in a more tender condition and more readily acted upon by the digestive juices. The highest factor was that for apple sauce, 95 per cent, and the lowest that for green corn, 60 per cent. The latter, however, was with only one subject, and if single experiments are considered, the results with Subject B. with potatoes and cabbage were both a trifle lower than that of corn. It is noticeable that the average for beets, 84 per cent, was above that for potatoes, 74 per cent; so also was that for cabbage, 77 per cent. In Constantinidi's experiments with potatoes, the digestibility of fibre was 78 per cent in one case, and 79 in the other. Weiske[1] made two experiments to determine the digestibility of fibre in a diet of celery, cabbage, and carrots, in one of which he found 63 per cent digested, and in the other 47 per cent.

Income and outgo of nitrogen. — In order to calculate the balance of income and outgo of nitrogen in these experiments, urine was collected in all but three of them, and its nitrogen content determined. Instead of the urine for the whole period of each experiment, however, only that for twenty-four hours, beginning at six o'clock on the morning of the last day of the study, was taken, as it was believed that by that time the body would have reached a stable condition as regards nitrogen assimilation and excretion for the given diet. Table III shows the elimination of urine for the day on which it was collected, and the percentage and amount of nitrogen in it, together with the gain or loss of nitrogen in the body as computed from the quantities of nitrogen in the food, fæces, and urine, that in the food and fæces being the average per day for the experimental period.

Experiments Nos. 1–6 were with Subject B. In the first one, with the large basal ration, there was a slight loss of nitrogen; in the

[1] WEISKE: Zeitschrift für Biologie, 1870, vi, p. 456.

second, with the large basal reduced one-third, the loss was considerable. In the next study, when cabbage was added to the reduced basal ration, the loss was somewhat less, but still large. In the fourth experiment, with potatoes added to the basal ration, there was a slight gain of nitrogen, but in the next two, with beets and apple sauce, the

TABLE III.

URINE DATA, AND INCOME AND OUTGO OF NITROGEN.

Experiment No.	Amount of urine.	Sp. gr. of urine.	Nitrogen in urine.	Nitrogen in urine.	Nitrogen in fæces.	Nitrogen in food.	Nitrogen gained (+) or lost (—).
	grams.		per cent.	grams.	grams.	grams.	grams.
1	638	1034	1.83	11.68	1.62	12.70	—0.68
2	614	1031	1.95	11.97	1.16	9.55	—3.58
3	693	1034	1.60	11.09	1.72	10.17	—2.64
4	931	1025	1.15	10.71	1.25	12 37	+0.41
5	926	1024	1.01	9.35	1.49	10.91	+0.07
6	1185	1017	0.69	8.18	1.52	9.69	—0.01
8	983	1028	1.14	11.20	1.02	13.59	+1.37
9	1100	1030	1.26	13.86	1.13	16.20	+1.21
11	824	1029	1.45	11.95	1.85	14.12	+0.32
12	1330	1014	0.57	7.58	2.10	14.85	+5.15
13	1416	1023	1.23	17.42	1.74	26.46	+7.30
14	971	1022	1.33	12.91	1.30	14.87	+0.66
15	961	1027	1.54	14.80	1.31	18.20	+2.09

difference between income and outgo of nitrogen was practically nil. In the two studies with Subject M. in which urine was collected, Nos. 8 and 9, with cabbage and potatoes respectively, there was almost the same gain of nitrogen in both. In the studies with Subject W. there was also a gain, which was small in the experiments with cabbage and apple sauce, but large in the others. On the whole, except in the study of cabbage with Subject B., the diet containing the vegetables seemed to supply the bodily needs of the different subjects for protein.

Conclusion.

So far as sources of protein or fat are concerned, the vegetables included in these studies may be considered as of little value. They do, however, contain carbohydrates, which the results of these and other experiments indicate to be quite well digested and absorbed; and they may, therefore, be considered as of value as sources of energy, a large proportion of which appears to be available to the body. The chief value of many vegetables, however, is perhaps aside from the nutrients or energy they furnish; they add a pleasing variety and palatability to the diet, supply organic acids and mineral salts, and give the food a bulkiness that seems to be of importance in its mechanical action in maintaining a healthy activity of the alimentary tract. Possibly the result of these conditions is a favorable influence upon the digestion of other food eaten with the vegetable; at least such an effect was suggested by the results of some of these experiments. For instance, in the studies with Subject B., with potatoes and with apple sauce added to the basal ration, the digestibility of the total ration, including such material, was noticeably higher than that of the basal ration alone.

ON THE ACTION OF SALINE PURGATIVES IN RABBITS AND THE COUNTERACTION OF THEIR EFFECT BY CALCIUM.[1]

By JOHN BRUCE MacCALLUM.

[From the R. Spreckels Physiological Laboratory of the University of California.]

I. The Mechanism of the Action of Saline Purgatives.

IN attempting to explain the action of saline purgatives, Schmiedeberg[2] states that these salts are absorbed with difficulty, and hence reach the lower parts of the intestine unchanged. In the large intestine, according to his theory, the salts prevent the fæces from becoming compact by inhibiting the absorption of fluids from the lumen. This hypothesis is supported by Wallace and Cushny,[3] who add that the absorption of fluids from the intestine is retarded especially by the salts of those acids which tend to form insoluble salts with calcium. Hofmeister[4] found that gelatin plates absorb less water when soaked in solutions of sodium sulphate, tartrate, citrate, etc., than they do when soaked in chlorides or bromides. Schmiedeberg's theory of the action of purgatives has been widely accepted. The older idea of Liebig that the salt solution attracts fluid from the blood into the lumen of the intestine on account of its osmotic pressure has long been abandoned. The purgative action of the salt solution does not increase with an increase in its concentration (Buchheim). Loeb,[5] while not denying the possibility of the inhibiting action of the saline purgatives on absorption, states

[1] A preliminary report of the results of these experiments was published in the University of California Publications, Physiology, May 25, 1903, Vol. i, No. 2, p. 5.

[2] Schmiedeberg: Arzneimittellehre, Leipzig, 1883.

[3] Wallace and Cushny: This journal, 1898, i, p. 411.

[4] Hofmeister: Archiv für experimentelle Pathologie und Pharmakologie, 1888, xxv, p. 1.

[5] Loeb: Decennial Publications of the University of Chicago, 1902, p. 10.

that these salts are identical with those which produce contact irritability, muscular twitchings, and hypersensitiveness of the nervous system. Further, he suggests that the increased peristalsis may be due to an increase in the irritability of the nerves and muscles of the intestine.

In order to decide this question, and to render more clear the action of saline purgatives, I have made a series of experiments on rabbits, testing a number of salts, including sodium citrate, sulphate, tartrate, oxalate, phosphate, and fluoride, barium chloride, and magnesium sulphate. It was found that the effects of the drugs could be most satisfactorily studied by exposing the intestines and observing them directly in animals under the influence of morphine. Subcutaneous injection of 5 c.c., 1 per cent solution of morphine proved sufficient for surgical anæsthesia, without affecting the intestinal movements in the rabbits which were used. These weighed on an average twelve hundred grams. In addition to this method, many experiments were made in which animals were kept in separate cages, and the amount and character of the fæces observed during several hours after the administration of the salts.

It was found that *all those salts which act as purgatives when introduced into the stomach or intestine, have the same action when injected subcutaneously or intravenously.* The injection of 1–2 c.c. $\frac{m}{8}$ sodium citrate solution [1] into the jugular vein of a rabbit causes a striking increase in the peristaltic movements. This activity begins in from one to two minutes after the injection. Loops of the intestine which before the injection lay quiet and collapsed, are set in active motion. They become rounded and prominent, and seem to occupy a greater volume. The fact that the animal is under the influence of morphine does not interfere to any extent with this action. Claude Bernard [2] has stated that sodium sulphate introduced into the veins acts as a purgative; and that the same action is obtained by subcutaneous injection of magnesium sulphate.

When introduced into the intestine or stomach a much larger quantity of the salt is required to produce an equal effect, and the action

[1] The salt solutions were made up with distilled water as fractions of molecular solutions. Thus $\frac{m}{8}$ sodium citrate solution means $\frac{1}{8}$ molecular weight of the salt in grams (including water of crystallization) dissolved in 1000 c.c. water.

[2] Claude Bernard: Leçons sur les effets des substances toxiques et médicamenteuses, Paris, 1857.

takes place only after an interval of from ten to fifteen minutes. By piercing the wall of the intestine or stomach with a hypodermic needle, and forcing 5–10 c.c. $\frac{m}{8}$ sodium citrate solution into the lumen, a similar increase in peristalsis is brought about. The movements begin about ten to fifteen minutes after the injection, not only in the loops of intestine which contain the solution, or the loops near the stomach, but simultaneously in all parts of the intestine.

When given subcutaneously, these salts do not act immediately, but only after an interval of ten to fifteen minutes. By this method usually a fairly large quantity, *e.g.*, 10 c.c. $\frac{m}{8}$ sodium citrate solution is necessary to produce increased peristaltic movements. When the actual passage of fæces is observed, relatively large doses also are necessary. No appreciable effect is obtained with less than 10 c.c. $\frac{m}{8}$ sodium citrate solution given subcutaneously. A number of control animals were kept in each case, and the average weight and character of the normal fæces carefully determined. During the first six hours following the injection of the salt, the fæces were collected and weighed. The purgative effect usually takes place during the first hour. Considerable variation in the action is to be observed in different rabbits; but with subcutaneous injection of the salt, there is constantly a marked increase in the weight of the fæces varying from two to six times the average normal weight. The fæces are sometimes of a semi-fluid character, a fact which is the more striking because the normal fæces of rabbits are drier and more definitely formed than the dejecta of most other animals.

Schmiedeberg's theory was an effort to explain this increase in the fluid contained in the fæces following the administration of a saline purgative. That the prevention of the absorption of fluids from the intestine is not the main cause of the production of fæces containing much fluid is shown by the following facts: In addition to the increase in peristalsis caused by the administration of the saline purgatives (whether subcutaneous, intravenous, or intraintestinal) *there is to be observed also a more or less marked increase in the secretion of fluid into the intestine.* A short time (twenty to thirty minutes) after the salt is given there is usually found in the intestine a considerable quantity of a clear yellow fluid. This does not resemble bile. The collapsed loops of intestine, in addition to being set in active squirming motion, become gradually filled with fluid after

the purgative is administered.[1] The actual passage of fæces takes place usually within an hour after the intravenous injection of the purgative. The exact nature of the fluid secreted into the intestine has not been studied, and the details of its secretion remain to be investigated. It was noticed also in a number of cases that an increased flow of saliva and urine occurred when one of these salts was introduced into the body. These problems of secretion are still to be studied. After the subcutaneous administration of sodium fluoride solution, the salivation in two or three cases was so marked that the saliva fell in drops to the floor. In overdoses of sodium citrate, the same phenomenon was observed in several cases; while it is well known that barium chloride causes salivation. Repeated urination is a fairly constant accompaniment of the subcutaneous and intravenous injections of all of these salts.

In connection with the production by these purgative salts of an increased flow of fluid into the intestine, together with salivation and increased urination, it is interesting to note the subcutaneous and intravenous administration of pilocarpine and physostigmin as purgatives by veterinarians. These drugs act primarily upon the glands of the body, and no doubt owe their purgative action to the production by them of increased secretion of fluids into the intestine.

The action of the various salts which act as purgatives when administered subcutaneously or intravenously varies in intensity; but it is difficult to make a definite list in which they can be placed in order of increasing strength. Barium chloride is the most powerful of all. The action is rapid, and with very small doses marked purgative effects are obtained. It is well known among veterinarians as a powerful purgative, and is commonly administered by them intravenously. An intravenous injection of 0.75–1 gram is sufficient to purge a horse weighing one thousand pounds. The intensity of action of sodium citrate, fluoride, sulphate, tartrate, oxalate, and phosphate decreases approximately in the order named. Magnesium sulphate, when given subcutaneously, is no less active than sodium sulphate, but has a somewhat more poisonous effect than sodium sulphate or sodium citrate. Large subcutaneous doses often cause death in a very short time in rabbits, while equal doses of sodium sulphate seem to be harmless. As a subcutaneous purgative, it is not to be

[1] As quoted by SCHMIEDEBERG (*Loc. cit.*), BUNGE has stated that sodium phosphate in rabbits causes an increase in the fluids contained in the alimentary canal. I have been unable to find BUNGE's original statement.

recommended. Barium chloride also produces unfavorable symptoms. Rabbits often do not recover from a relatively small dose. Sodium salts, however, such as the sulphate, citrate, tartrate, etc., may apparently be given subcutaneously with impunity. With regard to the common use of barium chloride by veterinarians, attention must be called to its dangerous nature, and to the possibility of the occurrence of serious after-results.

In addition to the purgative effects of these salts, there is produced by the injection of sodium citrate, tartrate, fluoride, oxalate, and phosphate a condition of extreme hypersensitiveness of the muscles and the nervous system, which is entirely analogous to that described by Loeb[1] in isolated muscles, motor nerves, and the skin of frogs. The muscular twitchings and increase in nervous excitability which Loeb caused in isolated muscles and in living frogs by means of these salts, I have been able to produce to a marked degree in rabbits by the subcutaneous injection of the same solutions. Subcutaneous injection of 10 c.c. $\frac{m}{1}$ sodium citrate solution produces within half an hour well-marked twitchings of the muscles in all parts of the body, more noticeable in those of the gluteal region. These are accompanied by tetanic contractions of the limbs, and in some cases by general convulsions of varying intensity. There is always a hypersensitiveness of the skin, and all the reflexes are much exaggerated. One of the first effects to be noticed is an incoordination of the movements of the hind limbs. If the animal is held up by the ears, the feet tremble, and, if touched, the hind limbs jerk away violently, or become rigid. The muscular twitchings appear immediately at the place of injection, but only after twenty to twenty-five minutes on the opposite side of the body.

In animals to which repeated small doses of sodium citrate are given, a chronic state of hypersensitiveness may be produced. Daily doses of 5 c.c. $\frac{m}{1}$ sodium citrate solution were given to a rabbit throughout one month, and then discontinued. A condition of marked hypersensitiveness persisted for four weeks after the last dose was given. In such an animal muscular twitchings may be produced on the side of the body opposite to the injection in as short a time as two minutes, while in a normal animal it takes twenty to twenty-five minutes.

To recapitulate, then, those salts which produce muscular twitch-

[1] LOEB: *Loc. cit.*

ings and hypersensitiveness of the nervous system, produce also increased peristalsis, and increased secretion of fluid into the intestine. They produce this purgative effect not only when introduced into the intestine, but also when injected subcutaneously or intravenously. *The presence of the salt in the lumen of the intestine is therefore not necessary for its cathartic action.* When introduced into the intestine, it takes much longer to act, and requires a much larger dose than is needed when injected into the blood. This seems to indicate that *the salt must first be absorbed into the blood before it can act on the intestine.* The essential feature in the action of saline purgatives is not their presence in the lumen of the intestine, but their absorption into the blood, and the production by them of a condition of hypersensitiveness of the nervous system controlling the intestine. It is difficult to say which part of the nervous system is specially affected. It is possible that the muscle and gland cells themselves are influenced; and it seems justifiable to suggest that the action of these salts is not a specific one, but that they cause a more general hypersensitiveness. It is difficult to disprove Schmiedeberg's theory, that the absorption of fluids from the intestine is retarded by these salts; but it is certain that the main factor in the production of fluid or less solid fæces is the increased secretion of fluid into the intestine. Since an increased secretion is caused by the absorption of the salt, the methods commonly employed in determining the rate of absorption of fluids from the intestine must be defective because they consist in measuring the fluid contained in a certain loop before and after the introduction of a measured quantity of salt solution. This quantity would remain relatively large, not because of a retardation of the absorption, but on account of the increased flow of fluid into the intestine caused by the absorption of the salt.

II. The Effect of Calcium in Opposing the Action of Saline Purgatives.

It has already been shown by Loeb that the muscular twitching and hypersensitiveness of the nervous system produced by sodium citrate, etc., may be inhibited by calcium salts. I have found that a complete analogy exists between this action and the production and inhibition of peristaltic movements in the intestine. *Active peristalsis produced by the intravenous injection of a minimal dose of any purga-*

tive sodium salt can be almost entirely suppressed by the subsequent injection of an equal quantity of $\frac{m}{8}$ calcium chloride solution. This counteracting effect takes place within one to two minutes after an intravenous injection, and in ten to twenty minutes after a subcutaneous injection, or the introduction of the solution into the intestine or stomach. A much larger quantity is required when introduced subcutaneously or into the alimentary canal. To illustrate this effect, one of a large number of experiments may be cited. To two similar rabbits, equal doses of morphine were administered. The abdominal cavities were opened, and in each case $1\frac{1}{2}$ c.c. $\frac{m}{8}$ sodium citrate solution were injected into the jugular vein. Active peristalsis set in in both animals. After five minutes $1\frac{1}{2}$ c.c. $\frac{m}{8}$ calcium chloride solution were injected into the jugular vein of one animal, while the other was left without further treatment. Almost immediately the peristaltic movements of the intestine of the animal which received the calcium chloride ceased. Those of the other animal continued. Usually after the injection of calcium the intestines remain motionless for one-half to one hour, while in some cases slight peristaltic movements begin after five to ten minutes. This depends largely upon the relative amounts of citrate and calcium chloride solutions which have been administered. It is possible after the movements have been entirely inhibited by calcium chloride to make them active again by a second injection of one of the purgative sodium salts. It is unimportant whether the intestines are set in motion by sodium citrate, sulphate, tartrate, phosphate, or oxalate. The calcium chloride has the same inhibiting effect in all cases. The peristaltic movements produced by barium chloride are usually not stopped by the administration of calcium.

As suggested by Dr. Loeb, the salts which produce rhythmical contractions in voluntary muscle are those which are liable to decrease the concentration of the free calcium ions. The same is true of the salts which act as purgatives; and the hypothesis that their action is due to the removal of calcium from the tissues, or from the fluids bathing them, is a plausible one. It is certain that the subsequent addition of calcium to the tissues restores them to the condition in which they existed before the injection of the purgative salt, and entirely counteracts the purgative effect. It is only on these grounds, and from the fact that these salts in some cases precipitate calcium, that it is possible to state that the purgative action is caused by the removal of calcium from the tissues.

It is not possible to make the general statement that the anion stimulates and the kation inhibits the muscular contractions. In a number of salts it is clearly the anion which causes the purgative action and the muscular contractions, *e.g.*, sodium citrate, sulphate, tartrate, fluoride, etc.; while in other cases it is equally clear that the kation has the same stimulating action, *e.g.*, barium chloride. The kation of calcium chloride on the other hand has an inhibiting effect. That this action also is not dependent upon the valency of the ions is shown by the fact that barium chloride, sodium fluoride, and sodium citrate all have the same effect, while barium chloride and calcium chloride have effects which are directly opposed to one another. Moreover, the effects produced by magnesium and sodium sulphate on the intestine are similar.

Little can be said at present concerning the practical application of these facts in the treatment of disturbances of the human intestine. *The administration of calcium, however, seems rational in cases of persistent diarrhœa, especially in those cases accompanying hysteria or any disturbance of the nervous system.* And since calcium itself has an irritating action when given in large doses, the treatment would seem to be contraindicated in cases in which the diarrhœa is caused by an inflammation of the mucous membrane of the intestine. The empirical use of calcium, usually in the form of chalk, is commonly resorted to in cases of diarrhœa, its action being explained by the statement that it reduces the acidity of the intestinal contents. But it is evident that it can only act through the calcium, since calcium chloride is more efficacious even than chalk.

In addition to this, the fact that a chronic hypersensitive condition of the muscular and nervous tissues may be brought about in rabbits by the continued administration of salts which tend to diminish the concentration of calcium ions, suggests that the addition of calcium to the tissues might be of service in the treatment of similar conditions in human beings. Those conditions which most nearly resemble that produced in rabbits are hysteria, neurasthenia, and the allied states. Whether there is in reality any analogy, and whether calcium will prove of benefit in such cases, must be the subject of clinical investigation.

The question arises also as to the possibility of administering purgatives to human beings subcutaneously or intravenously. Although in general such methods would be contraindicated, certain cases might arise in which it would be of distinct advantage. The present experi-

ments seem to indicate that subcutaneous or intravenous administration of some of the salts, especially sodium citrate, might be safely resorted to. Neither barium chloride nor magnesium sulphate should be given in this way.

III. Conclusions.

1. In general, the saline purgatives act not only when introduced into the intestine, but also when injected subcutaneously or intravenously.

2. The intensity of their action is greatest with barium chloride and decreases approximately in the following order: barium chloride, sodium citrate, fluoride, sulphate, tartrate, oxalate, and phosphate.

3. The purgative action of these salts is caused, first, by an increase in peristalsis, and, second, by increased secretion of fluid into the intestine, both of which can be directly observed.

4. Although I have not specially studied the secretion of other glands, I have noticed that in a number of cases an increased flow of saliva and urine occurred when these salts were introduced into the body.

5. Intravenous injection of 1–2 c.c. $\frac{m}{8}$ solution of these salts causes increased peristalsis within one minute. When introduced into the intestine, it takes ten to fifteen minutes, and five times the amount to produce an equal effect.

6. This seems to indicate that even when these salts are introduced into the intestine, they must be absorbed into the blood before they can produce their purgative effect, and that they affect the intestine by increasing the irritability of the nerves and muscles, as Loeb has suggested. Their action in producing less solid fæces is not due to the prevention of the absorption of fluids from the intestine, but to the production of an increased secretion of fluid into the intestine.

7. By the continued administration of small doses of sodium citrate, a chronic condition of hypersensitiveness of the nervous system may be brought about in rabbits, which persists for a considerable time after the drug is discontinued.

8. By the injection of solutions of calcium chloride, the peristalsis caused by these salts can be entirely inhibited.

9. There is a perfect analogy between these actions and the pro-

duction and suppression of muscular twitchings and nervous hypersensitiveness.

10. The administration of calcium is, therefore, rational, especially in those cases of diarrhœa in human beings which accompany hysteria or nervous excitability of any sort.

The study of this subject was suggested to me by Professor Loeb, and it is a pleasure to thank him for the interest which he has taken in the experiments, and for many helpful suggestions.

THE CEREBRO-SPINAL FLUID IN HYDROCEPHALUS.

By ISADOR H. CORIAT.

[*From the Chemical Laboratory of the Worcester Insane Hospital.*]

ANALYSES of the cerebro-spinal fluid in hydrocephalus have been rather infrequent, because of the comparative rarity of this condition, and of the greater attention given to the anatomical rather than to the chemical findings. During the last few years the cerebro-spinal fluid has assumed great importance, from both a normal and pathological standpoint. Much remains to be cleared up, however, especially in regard to the form of proteid present, the nature of the reducing body, and the molecular concentration as established by the freezing point and its relation to the chloride content, before we shall know what analogy, if any, exists between this fluid and blood-serum. Unfortunately, the large amounts of fluid required for an investigation of this nature can only be obtained in hydrocephalic cases. So far as known, the fluid in this disease does not differ in its composition from that found in normal conditions. I have been able to discover only seventeen analyses of hydrocephalic fluid. The latest are two by Panzer[1] and one by Salkowski.[2] In these three the fluid was obtained at an early period of life. In Panzer's case, 455 c.c. and 180 c.c. respectively were obtained from two hydrocephalic fœtuses; in Salkowski's case, that of a young child, there was 1050 c.c. of fluid.

My case was that of a congenital hydrocephalic imbecile, forty-two years of age, who died suddenly, without any previous illness.

The fluid was obtained an hour and a half after death. The pressure was considerable, for, following an accidental slight rupture of the hemispheres during the removal of the brain, a stream of fluid escaped to the distance of almost six feet. In spite of this, however, 750 c.c. was obtained, although at least 150 c.c. was lost.

[1] PANZER, T.: Münchener medicinische Wochenschrift, August, 1899, p. 805.

[2] SALKOWSKI, E.: Festschrift für M. Jaffé, 1901, p. 263.

There was no spontaneous coagulation. The color was clear straw. After precipitation of the proteid by alcohol, the alcoholic extract was of a distinct amber color, and in the spectroscope there could be detected a broad band of absorption extending from F in green to beyond G. This coloring matter could not be extracted by shaking with amyl alcohol. The specific gravity of the fluid was 1012, and the reaction very slightly acid. A trace of lactic acid was present. There was a marked reduction with Fehling's solution. After removal of the proteid by heat coagulation, this reducing body was submitted to further study, with the following results. Fehling's test, positive. Nylander's test, positive. Molisch's reaction, deep purple ring. Trommer's test, positive. Sodium hydrate (with warming), deep yellowish-brown color. Fermentation test, positive after twelve hours. A control test with the same sample of yeast and distilled water gave no reaction after the same length of time. Phenylhydrazin test: 50 c.c. was heated in the water bath for an hour, with 1 gram phenylhydrazin and 2 grams sodium acetate, and allowed to cool slowly. There was obtained an abundance of long yellow needles, arranged in sheafs, resembling phenylglucosazon and having a melting point of 205° C. Ferric chloride, no reaction. Hydrochloric acid — phloroglucin — negative. Orcin, negative. Ammoniacal silver nitrate, strong reduction to metallic silver. The amount of this reducing body (in terms of dextrose) was 0.917 gram per 1000 c.c.

Pyrocatechin, cholesterin, and cholin were absent. There was a trace of fat. Urea was present, the amount being 0.750 gram per 1000 c.c. The freezing point was –0.65° C., and the relation $\frac{\Delta}{\mathrm{NaCl}}$ per cent was 0.97. The total proteid was 1.180 grams per 1000 c.c. Fibrin, nucleoproteid, albumose, peptone, mucin, serum albumin, and fibrinogen (no coagulation at 56° C.) were absent, the entire proteid content present being in the form of serum globulin. It coagulated at 75° C., and was completely precipitated by saturating the fluid with magnesium sulphate in substance.

The ash on elementary analysis was found to consist of phosphorus, potassium, and sodium; calcium and magnesium were present in traces, while iron was absent. The nitrogen of the proteid and urea amounted to 0.5312 gram per 1000 c.c. The relation $KCl : NaCl$ was 1 : 10.28– of $K_2O : Na_2O$ was 1 : 8.5.

Tests for a proteolytic ferment showed no digestion of fibrin after

twenty-four hours, either with or without the addition of hydrochloric acid. A diastatic ferment, however, was present, but it was inactive in a neutral or acid medium, and only became active after the fluid was made slightly alkaline with sodium carbonate. It carried starch digestion through the various dextrines to the final production of maltose. The tabulated results of the quantitative analysis follow:

Substance.	Parts per 1000 c.c.	Substance.	Parts per 1000 c.c.
Water	983.8	Potassium chloride . . .	0.6559
Fixed solids	16.2	Potassium oxide	0.4143
Volatile substances . . .	10.6	Sodium oxide	3.5227
Ash	5.6	Reducing body	0.917
Total proteid	1.180	Total nitrogen	1.260
Extractives and salts . .	15.020	Urea	0.750
Chlorides	9.5	Phosphoric acid	0.090
Sodium chloride	6.6441		

The coloring matter was a lipochrome, resembling serum lutein (lutein of Kühne), but differed from it in not being soluble in amyl alcohol, and in presenting before the spectroscope one broad band of absorption, instead of two narrow ones, although occupying the same relative position in regard to the lines. The reducing substance was of the hexose group, as it failed to give the characteristic reaction for pentoses with orcin or hydrochloric acid — phloroglucin. In all probability it was dextrose, as it responded to all the usual tests, reducing the salts of the various metals in alkaline solution, producing fermentation with yeast, and the osazon, in both crystalline form and melting point, exactly resembled phenylglucosazon. Panzer's second case contained glucose, and Salkowski in his last analysis speaks of the fluid as containing a fermentative sugar. This is in direct opposition to Halliburton's claim, that the reducing substance in cerebro-spinal fluid is pyrocatechin, for a careful testing of the fluid in my case for this substance, using the method recommended by Halliburton, failed utterly to detect it. The characteristics on which he bases his claim, namely, the non-production of alcoholic fermentation and the failure to reduce bismuth salts, were certainly

absent in this analysis. In large amounts of fluid obtained from cases of general paralysis, I also found this reducing body responding to the tests detailed above. At one time I believed that it was closely related to the nitrogenous glucosides (cerebrins), but further investigation along this line, using the method for isolation of the cerebrins recommended by Koch, has shown this to be untenable. The amount of dextrose present was greater than that contained in blood. The chloride content is greater than in normal serum, and the larger part of it is in combination with the univalent radicals, sodium and potassium. The molecular concentration, as shown by the depression of the freezing point, is also greater than in normal serum or in defibrinated blood, for Hamburger has shown the freezing point of the latter to be that of blood-serum. This lower freezing point is due to the greater quantity of dissociated salts, especially the chlorides. A proteolytic ferment was absent, but, as in the blood, there was present a ferment capable of hydrolyzing starch into sugar (maltose). The proteid consisted entirely of globulin. Its coagulation point was the same as that of serum globulin, and furthermore, it was completely precipitated by magnesium sulphate in substance. There was no cholin, thus proving the absence of any recent active degeneration in the central nervous system.

ON THE TIME RELATIONS OF PROTEID METABOLISM.[1]

By P. B. HAWK.

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CONTENTS.

Historical Summary.

INVESTIGATIONS[1] upon the time relations of proteid metabolism seem to have been inaugurated about 1855 by Becher.[2] About this date Lehmann[3] writes, " It is noteworthy that very soon after the ingestion of food rich in nitrogen an increase in the urea excretion occurs, and five-sixths of the nitrogen contained in the food is often eliminated in twenty-four hours, as urea." Lehmann does not appear to have made accurate observations upon the in-

[1] It is not the author's purpose, however, to give a complete review of this subject, but rather to report the results of his experiments, with only such references to other work as seem called for in this connection.

[2] Becher: Zeitschrift für rationelle Medicin, 1855, vi, p. 249.

[3] Lehmann: Lehrbuch der physiologische Chemie, second edition, i, p. 163.

crease in the urea output from hour to hour, neither does he draw any conclusions from his observations.

Karl Voit,[1] in 1857, described experiments made with dogs fed on different quantities of lean meat. He found that the urea excretion was increased the first hour after the meal, reaching its maximum in the seventh hour. His figures indicate that in twenty-four hours after ingestion, an amount of nitrogen equivalent to that contained in the food was excreted mainly as urea.

Winternitz[2] in a series of experiments carried out upon himself reached the conclusion that the maximum urea excretion occurred sometimes in the third, and sometimes in the fourth hour after normal feeding. In one experiment this investigator took 40 c.c. "rum" with an ordinary meal, determined the urea content of the urine in hourly periods thereafter, and found the maximum excretion during the first hour (8–9 A.M.). The food was not analyzed, and there were no periods with normal diet for comparison.

J. W. Paton[3] in a series of investigations with himself and Gamgee as subjects, observed, among other things, the effect of severe mental work upon metabolism. He found that with mental work the amount of urine and its nitrogen content increased, and the phosphoric acid content slightly decreased. On returning to comparative rest after the severe mental work, the nitrogen excretion was greatly diminished in every case, while the phosphoric acid excretion was slightly increased with one subject and unchanged with the other. The author holds that urea has no relation to mental work except in so far as the latter influences the excretion of water. Under this "perverted nervous action" a general wash-out takes place, and the urea is therefore increased.

Forster,[4] in an experiment of twenty-four hours' duration, upon a man, collected the urine in six four-hour periods. The experiment began with a breakfast of meat supplying eighteen grams of nitrogen; no other food was taken. The largest excretion of nitrogen was during the second, and of phosphoric acid during the first, four-hour period. The author shows that when Voit's results are tabulated in four-hour periods, the maximum excretion of nitrogen is also in the second four-hour period, or from five to eight hours after the ingestion.

[1] VOIT: Physiologische chemische Untersuchungen, Augsburg, 1857, p. 42.

[2] WINTERNITZ: Wiener medizinische Jahrbücher, 1864, xx, p. 3.

[3] PATON, J. W.: Journal of anatomy and physiology, 1871, v, p. 285.

[4] FORSTER: Zeitschrift für Biologie, 1873, ix, p. 383.

Panum[1] and Carl Philip Falck[2] made valuable experiments with dogs. Those of Panum give interesting data concerning the influence of fat upon the excretion of urea. Upon a diet of five hundred grams of beef the dog excreted its maximum of urea in from two and one-half to five hours after the ingestion, whereas when thirty grams of pork fat were added to a like amount of beef, the maximum urea excretion did not occur until the sixth or eighth hour thereafter. When one hundred and fifty grams of rye bread were added to the latter ration, the maximum urea excretion occurred after only one and one-half to four hours. Unfortunately the periods of collection of urine were irregular, and the results are consequently less definite than might be desired. Falck fed his dogs quantities of beef, ranging from one-half to one and one-half kilograms, and made hourly collections of urine thereafter by use of a catheter. The maximum excretion of nitrogen occurred during the seventh to twelfth hour after the ingestion of the food, being in general later as the quantity of meat was larger.

Oppenheim[3] in experiments upon himself found the maximum of urea generally from four to seven hours after somewhat irregular meals.

Feder[4] conducted an extensive series of experiments upon the time relation of proteid metabolism with dogs. In each case one-half kilogram or one kilogram of meat was taken at the beginning of the experiment. The maximum nitrogen excretion was reached in four to six hours with one-half kilogram and in six to eight hours with one kilogram of meat. The excretion of sulphur as sulphate in the urine reached its maximum in one experiment in four to six hours and in another in two to four hours. In every case the excretion of phosphorus as phosphate rose more rapidly than that of nitrogen and reached its maximum earlier (in two to four hours); a rapid decline soon followed. The author found that when five grams of sodium chloride were added to the meat diet the excretion of nitrogen was accelerated and increased. Feder also made a series of experiments with a diet containing fat, supplied in 150–200 grams of

[1] PANUM: Nordiskt medicinskt Arkiv, 1874, Jahresbericht für Thierchemie, 1874, iv, p. 361.

[2] FALCK, C. P.: Beiträge zur Physiologie, Hygiene, Pharmacologie, und Toxicologie, i, Stuttgart, 1875.

[3] OPPENHEIM: Archiv für die gesammte Physiologie, 1880, xxiii, p. 446.

[4] FEDER: Zeitschrift für Biologie, 1881, xvii, p. 531.

bacon, added to 400–500 grams of beef. The maximum nitrogen excretion occurred in two to six, that of sulphur in one to two, and that of phosphorus in one to four hours after taking the food. There were no analyses of the food and no central periods.

The results of such comparatively recent investigations as those of Rosemann,[1] Tschlenoff,[2] Riazantseff,[3] Veraguth,[4] Roeske,[5] and Graffenberger[6] have already been sufficiently reviewed in this journal.[7]

In an inquiry regarding the excretion of uric acid, François Marès[8] gives data regarding the elimination of nitrogen. In nearly all of his experiments the urine was collected hourly and the nitrogen content determined. The subjects were boys and men ranging in age from thirteen to forty-five years. They received amounts of beef varying from one-half to one and one-half kilograms at a single meal. The maximum nitrogen excretion appeared in six to nine hours after the ingestion. The author concludes that uric acid excretion is a "function of age and individuality," and that the nitrogen excretion is variable, depending principally upon the quantity of nitrogenous food ingested, and is independent of the age and individuality of the subject.

Gley and Richet[9] in experiments with themselves on uniform diet found the maximum urea excretion three to four hours after the ingestion of the food. The conditions were, however, somewhat irregular and the data by no means complete.

Sondén and Tigerstedt[10] in the discussion of the results of an inquiry on "Die Respiration und der Gesammtstoffwechsel des Menschen," refer to the nitrogen excretion in the urine during different hours of the day, but the point of maximum excretion is difficult to determine, as the different days are not divided alike.

[1] Rosemann: Archiv für die gesammte Physiologie, 1896, lxv, pp. 343–392.

[2] Tschlenoff: Correspondenz-Blatt für Schweizer Aerzte, 1896, xxvi, p. 65.

[3] Riazantseff: Archives des sciences biologiques, 1895, iv, pp. 895–896.

[4] Veraguth: Journal of physiology, 1897, xxi, p. 112.

[5] Roeske: Ueber den Verlauf der Phosphorsäure-Ausscheidung beim Menschen. Dissertation, Greifswald, 1897.

[6] Graffenberger: Zeitschrift für Biologie, 1892, xxviii, pp. 318–344.

[7] Sherman and Hawk: This journal, 1900, iv, p. 26.

[8] Marès: Archives slaves de biologie, 1887, iii.

[9] Gley et Richet: Comptes rendus de la société de biologie, Paris, 1887, iv, p. 377.

[10] Sondén und Tigerstedt: Skandinavisches Archiv für Physiologie, 1895, vi, 1.

Hopkins and Hope,[1] in experiments with men, found the maximum nitrogen excretion in general during the third or fourth hour after the ingestion of nitrogenous food.

Sherman and Hawk,[2] in experiments upon themselves in this laboratory, observed the effects of sudden increase of nitrogen in the food from eating extra amounts of lean meat at breakfast. The maximum rate of nitrogen excretion occurred in six to nine hours after the extra proteid was ingested; the rise and fall of the sulphate excretion was nearly parallel to that of nitrogen. The course of the phosphate excretion was entirely different from that of either nitrogen or sulphate.

Herfeldt,[3] Johansson,[4] Bert,[5] Kaupp,[6] and Van Noorden[7] have also conducted investigations upon nitrogen metabolism which are closely related to those of the present paper.

Methods.

Purpose and plan of the experiments here reported. — The aim of the present investigation has been to determine: (1) The length of time elapsing between the ingestion of large amounts of proteid food and the excretion of increased amounts of nitrogen, sulphur, and phosphorus in the urine. (2) The balance of income and outgo of nitrogen, sulphur, and phosphorus. (3) The relation between the nitrogen content of the urine and the heat of combustion of its water-free substance.

A preliminary period of four days (Period I) was passed on a diet containing 14.86 grams of nitrogen and about 2900 calories of energy.

On the fifth day (Period II) at breakfast, a portion of the normal diet having a nitrogen content of 2.46 grams was replaced by a proteid food containing 12.60 grams of nitrogen. In other words, the

[1] Hopkins and Hope: Journal of physiology, 1898, xxiii, p. 271.

[2] Sherman and Hawk: *Loc. cit.*

[3] Herfeldt: Mittheilung aus der Würzburger medicinische Klinik., 1885, i, p. 61, Centralblatt für die medicinische Wissenschaften, 1885, xxiii, p. 515.

[4] Johansson: Skandinavisches Archiv für Physiologie, Leipzig, 1898, viii, pp. 85–142.

[5] Bert: Jahresbericht für Thierchemie, 1879, ix, p. 291.

[6] Kaupp: Archiv für physiologische Heilkunde, 1856, p. 554.

[7] Van Noorden: Pathologie des Stoffwechsels, 1893, Physiologische Theilung, p. 45.

amount of nitrogen ingested at the meal was increased by 10.14 grams. During the remainder of that day, and for the four days following (Period III), the constant diet of the preliminary period was again maintained. Urine was passed every three hours during the day, beginning at 6.30 A. M., and in a nine-hour period at night, beginning at 9.30 P. M.

The total nitrogen, sulphur, and phosphorus were determined in foods and fæces. The total nitrogen, the sulphur as SO_3, and the phosphorus as P_2O_5 were determined in the urine.

Diet. — The foods used in the constant diet were soda crackers, butter and whole milk (see Table I). The extra proteid of the fifth

TABLE I.

DIET: AMOUNT AND COMPOSITION.

Food.	Dry matter.	Fat.	Ash.	N.	SO_3.	P_2O_5.	Heat of combustion per gram.	Amt. of food eaten per day.	
								Normal days.	Day of extra proteid ingestion.
	per cent.	per cent.	p. cent.	per cent.	p. cent.	p. cent.	sm. cal.		
Beef (partially dried)	96.40	7.70	2.38	14.00	2.24	1.87	5585	....	225 (fresh)
Crackers	90.53	6.20	1.92	1.84	0 28	0.24	4216	300	250
Butter	91.44	....	2.67	(1.2% casein)	....	....	8018	60	60
Milk (partially dried)	96.52	34.09	4.38	4.41	1.21	2.02	5986	....	....
Milk (fresh)	....	Babcock = 4.3 Ether extract = 4.22	....	0.56	0.15	0.26	782	1650	1375
Beef (partially dried) = 40%. Milk (partially dried) = 12.71%.									

day was furnished by 225 grams of a specially prepared and very lean beef. Three meals were eaten each day: the first one at 6.30 A. M., the second at 12.30, and the third at 6.30 P. M. With one exception, explained below, each meal consisted of 550 grams of whole milk, 20 grams of butter, and 100 grams of soda crackers. Upon the morning when the extra proteid was to be taken, 275 grams of whole milk and 50 grams of crackers were replaced by 225 grams of beef. This substitution increased the ingested nitrogen for the whole day by 10.14 grams, though the energy of the diet was increased by only

80 calories, or from 3027 calories to 3107 calories. The particular feature in this case was that the extra proteid material was ingested all at one meal, without any further interruption of the usual conditions of the experiment.

The constant diet became at no time distasteful, and seemed eminently fitted for the needs of the subjects. Feelings of hunger were entirely absent, and at the same time no unpleasant sensation, such as excess of food sometimes causes, was experienced. When the added beef was taken, however, Subject R. found a slight difficulty in consuming the entire two hundred and twenty-five grams. This was due, no doubt, mainly to the fact that the only method feasible for preparing the beef before eating, was to heat it upon a water-bath. No frying-pan or spider could be used, for with either of these a loss of material would have occurred; and the quantity of butter in the diet was too small to grease the pan. The prepared beef was quite dry and tasteless, and the subjects found the quantity of saliva secreted rather inadequate for the consumption of the large amount of unmoistened beef.

Subjects. — The subjects of the experiments here reported were two men twenty-five years old in normal health. Both were wholly unaccustomed to the use of alcoholic beverages. One was accustomed to use moderate amounts of coffee and tobacco, but during the time of the experiments and for a number of days previous he abstained from both. The weights of the men, without clothing, at the beginning of the experiment were: H. 56.2 kg. and R. 60 kg., and at the end they were: H. 56.4 kg. and R. 59.2 kg. Thus H. had gained 0.2 kg. during the nine days, whereas R. had lost 0.8 kg. The weights were taken on the first and last days, about 6.30 A.M., before any food was eaten, and immediately following micturition and defæcation. The attempt was made to have the contents of the alimentary canal and bladder as small and nearly alike as practicable at the times of weighing.

Daily schedule. — The subjects rose at 5.30 A. M., and as they lived near the laboratory they were ready for work at 6 A. M. The time up to 6.30 A. M. was passed in preparing breakfast and in beginning the analytical work of the day. Immediately after breakfast the urine samples completed at 9.30 the previous evening, and at breakfast time, were weighed, their specific gravity taken, and the urine aliquoited for composite samples, one-fifth of the urine of each subject being used for this composite.

The milk for the day was next thoroughly mixed, sampled, both for individual sample and for composite, and the fat determined by the Babcock test.

At 9.30 A. M. each subject drank 300 c.c. of water. Regular analytical work connected with the experiment proper or with investigations with the respiration calorimeter occupied the subjects from that hour until about 12.15 P. M. The midday meal was then prepared, and at 12.30 was eaten. From this hour until 3.30 P. M., regular analytical work and computation and tabulation of results occupied the subjects. At 3.30 P. M., 300 c.c. of water was again taken by each.

The time between 5 and 6 P. M. was devoted to active exercise, either brisk walking out of doors or, when the weather was unfavorable, light exercise in the university gymnasium near by. The subjects returned to the laboratory at about 6 P. M., prepared and ate their evening meal, and passed the remaining time to about 9.30 in the routine operations of weighing and analyzing samples, and calculating and putting into tabular form the results obtained from previous analytical work. At 9.30 P. M. each subject drank 300 c.c. of water and at about 10.15 P.M. went to bed.

All meals were taken in the laboratory building, thus rendering it unnecessary to leave the laboratory except for the usual afternoon exercise. In order to obviate any possible influence of nervous excitement upon metabolism, the subjects aimed to maintain absolutely normal conditions at all times and to eliminate as far as possible excitement of any kind.

Preparation of samples, methods of analysis, etc. — The crackers were purchased at a local grocery, and during the experiment were kept in a large tin can in order to insure constancy in the moisture content. Enough for the whole experiment were secured at one time, and sampled by taking crackers at random throughout the lot.

The butter was the best product of a neighboring creamery. A quantity sufficient for the whole experiment was obtained at one time, and the amount needed for each subject for each meal of the experimental period was accurately weighed and placed in a small ointment pot, before the experiment proper began. During this process small portions were taken occasionally for the sample.

The milk used in the experiments was a portion of the product of five cows, isolated from a large herd for this special purpose. The

beef was specially prepared and as free from fat as possible. In preparing the foods for analysis the usual method was followed.[1]

The fæces were dried at 100° C., then weighed, and ground, and the nitrogen, heat of combustion, etc., determined as with the foods (Table II). Separation of fæces was made by taking, at the beginning of the first meal of each period, two gelatin capsules each containing about 0.2 grams of powdered charcoal. Defæcations were normal throughout.

TABLE II.

AMOUNT AND COMPOSITION OF FÆCES.

Subject.	Period.[1]	Amount.	Dry matter.	Fat.	Ash.	Nitrogen.		SO_3.		P_2O_5.		Heat of combustion per gram.
		grams.	per cent.	per cent.	per cent.	p. cent.	grams.	p. cent.	grams.	per cent.	grams.	small cal.
II.	I	77.8	97.24	15.23	32 02	3 89	3.02	4.80	3.74	10.63	8.27	5207
	II	33.5	94.86	16.02	28.08	4.24	1.42	3.31	1.11	8.97	3.01	5524
	III	72.0	96.83	14.82	31.86	4.31	3.10	5.33	3.84	10.63	7.66	5186
R.	I	118.2	94.28	15.14	29.62	3.67	4.34	1.90	2.25	5.17	6.10	5468
	II	39.0	94.93	14.90	29.25	4.18	1.63	3.04	1.19	8.86	3.45	5493
	III	81.8	96.65	12.87	31.97	3.95	3.23	4.25	3.48	9.24	7.56	5160

[1] Period I = 4 days. Period II = 1 day. Period III = 4 days.

The fusion method[2] was used for the determination of total sulphur and phosphorus in the foods and fæces. The casein content of the butter was determined by dissolving out the fat with ether, weighing the residue, igniting the latter, and subtracting the weight of the ash, as recommended by the Association of Official Agricultural Chemists. The other methods have already been described in this journal.[3]

All analyses of the foods, as well as the urine and fæces, were made in duplicate. The daily analyses of milk and the analyses of urine

[1] See Bulletin 44, Office of Experiment Stations, United States Department of Agriculture.

[2] See HAWK and GIES, This journal, 1901, v, p. 493.

[3] SHERMAN and HAWK: *Loc. cit.*

for different periods of the day were controlled by analyses of composite samples. All specific gravity determinations, as well as all weighings of food, fæces, and urine, and all burette readings when convenient, were checked by two men in order to eliminate as nearly as possible any error which might arise from inaccurate readings.

TABLE III.

Analysis of Composite Urine Samples.

Subject.	Date (1900).	Amount.	Nitrogen.	SO_3.	P_2O_5.
		grams.	grams.	grams.	grams.
H.	Jan. 13	1414.2	13.040	1.856	2.603
	" 14	669.2	12.250	1.776	2.494
	" 15	726.6	13.007	1.870	2.433
	" 16	702.2	12.780	1.950	2.517
	" 17	1080.3	19.120	2.930	3.284
	" 18	740.3	14.435	2.080	2.525
	" 19	723.4	13.100	1.895	2.285
	" 20	768.6	13.260	2.047	2.724
	" 21	680.6	13.480	2.032	2.808
R.	Jan. 13	1664.6	14.450	2.086	2.838
	" 14	1041.2	14.212	2.085	3.130
	" 15	1553.6	15.536	2.280	3.320
	" 16	1428.8	14.430	2.285	3 087
	" 17	1599.6	21.435	3.432	3.556
	" 18	1081.0	15.566	2.265	2.761
	" 19	1269.4	14.726	2.288	2.690
	" 20	1218.7	14.260	2.145	3.027
	" 21	1655.3	13.800	1.997	2.823

Discussion of General Results.

The data relating to the diet will be found on page 120 (Table I). The amounts and composition of the fæces of the two subjects are given on page 123 (Table II). In Table III, page 124, will be found

the results of the analysis of the various composite samples of urine. Table IV, page 126, shows the volumes of urine for the various periods of the different days. For more convenient reference, the amounts of the nitrogen excretion for the various periods of the several days are condensed in Table V, page 128. Similar data for the excretion of SO_3 and P_2O_5 may be found in Tables VI and VII, on pages 134 and 137, respectively. The data of income and outgo of nitrogen may be found in Table VIII, page 139. Similar data for sulphur and phosphorus are given in Tables IX and X, on pages 141 and 142, respectively. Table XI, on page 143, summarizes the ratios of the nitrogen and heat of combustion of the composite sample of the urine of each day. The hourly excretions of nitrogen, sulphur, and phosphorus by the two subjects during the several experimental days are shown by curves in Figs. 1–4, pp. 127, 130, 133, 138.

Urine volume. — When we consider the fact that the daily consumption of liquid (water and milk) was the same for each subject, we are struck with the marked variation in the volume of the urine excreted by the two men. It is commonly stated that the total volume of the urine excreted during any definite period varies in a fairly regular degree according to the amount of liquid taken into the body during that period. In the case under consideration, we had two men of approximately the same age, weight and lung capacity, maintaining the same constant diet and drinking precisely the same amounts of water and milk daily; yet Subject R. excreted as an average 1400 grams of urine per day, while Subject H. excreted as an average but 850 grams (Table IV, page 126). The time being mid-winter, the subjects did not perspire very freely, and as far as could be judged the rate of sensible perspiration was about the same for each subject. It is difficult to explain why Subject H. should excrete but 60 per cent as much urine as Subject R. when the two men were engaged in exactly the same occupation, took the same amount of exercise at the same hour of the day, and worked side by side in the same room, thus securing like conditions as regards temperature, etc. As has been said (page 121), Subject H. gained 200 grams in body weight during the experiment, while Subject R. lost 800 grams in the same period, but this would account for only a very small part of the discrepancy between the urine volumes of the two men for the entire experimental period. Insensible perspiration may account for a portion of the variation in urine volumes, but it does not seem probable that Subject H. could have lost, in that way, 550 grams per day more than Subject R.

A similar phenomenon is observed in comparing by periods, the urine excretion of the two subjects. On every day except January 14, the volume of the urine excreted by Subject R. during the fourth period of the day (3.30 P.M. to 6.30 P.M.) was greatly in excess of that for any other period of the day for this subject, and also far greater than that excreted by Subject H. during this same period.

TABLE IV.

URINE VOLUME.

Subject.	Period.	Jan. 13.	Jan. 14.	Jan. 15.	Jan. 16.	Jan. 17.	Jan. 18.	Jan. 19.	Jan. 20.	Jan. 21.
		grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.
H.	I	98.2	90.7	112.4	100.6	130.0	102.3	108.1	81.2	88.3
	II	112.0	102.8	114.0	113.5	188.4	119.7	127.2	97.6	83.5
	III	85.5	77.2	82.1	84.1	151.2	91.3	82.3	92.0	83.5
	IV	436.6	79.9	86.1	74.8	140.2	82.9	76.9	77.7	78.9
	V	398.4	105.9	78.5	80.9	131.5	82.0	72.4	99.4	107.5
	VI	283.5	212.7	253.5	248.3	339.0	262.1	256.5	320.7	238.9
	Tot.	1414.2	669.2	726.6	702.2	1080.3	740.3	723.4	768.6	680.6
R.	I	149.4	161.5	133.2	120.8	125.2	130.4	142.6	101.7	125.8
	II	177.3	133.6	259.5	213.6	170.4	117.2	157.3	146.2	394.8
	III	190.5	173.5	156.3	119.0	183.5	85.7	109.0	132.8	235.2
	IV	725.7	151.6	399.0	549.4	405.4	214.1	411.6	178.7	507.1
	V	153.2	112.4	104.0	122.3	178.6	111.9	129.9	120.9	85.6
	VI	268.5	308.6	501.6	303.7	536.5	421.7	319.0	538.4	306.8
	Tot.	1664.6	1041.2	1553.6	1428.8	1599.6	1081.0	1269.4	1218.7	1655.3

The average excretion by Subject R. for this fourth period was 393.6 grams; while the average excretion by subject H. was but 126 grams, or only 32 per cent as great as the excretion by Subject R. Furthermore, examination of the tabulated data shows the volume of Subject R.'s urine for the fourth period to have been greater than the combined volumes for the third and fifth periods, on every day except January 14 and 20. On six of the nine experimental days the urine

volume of Subject R. for this fourth period was from 25 per cent to 43 per cent of the total twenty-four hours' urine. At 3.30 P. M., each subject drank 300 c.c. of water, as has been stated, and following this, at 5 P. M. came the customary exercise. Why these factors should so differently influence the urine volume of the two subjects, however, is not entirely clear.

Nitrogen excretion by Subject H. — The graphic representation of the nitrogen excretion by Subject H. will be found in Fig. 1, below, while the actual amounts of nitrogen excreted are stated in

FIGURE 1. — The nitrogen excretion by Subject H. The ordinates show the average hourly excretion in grams and the abscissæ the time in days.

Table V, page 128. The curve for this subject shows a general tendency for the nitrogen to be excreted in such a manner as to form two very well-defined maxima. This does not hold true for the day upon which the large amount of extra proteid food was ingested, nor for the two days immediately succeeding, but as soon as the normal level for the nitrogen excretion was again obtained (January 20) the two maxima reappear.

In every instance there was a very decided fall in the rate of excretion during the night period, and this was succeeded, in general, by a very perceptible rise during the first period of the day following, *i. e.*, just after the morning meal. This rise was without doubt due, at least in part, to the influence of the ingested food.

Taking into account the days which show the two maxima, it is noticed that in every case except one (January 15) the highest point reached by the day's excretion was in the fifth period or from 6.30 P. M. to 9.30 P. M. It will be remembered that just preceding this period (5 P. M.) the subjects were accustomed to take their daily

exercise. This light exercise can hardly be considered an important influencing factor, however, as it is very generally believed that any increase in the nitrogen excretion due to muscular work does not appear before the following day.[1] The evening meal, at 6.30 P. M., very likely had an influence upon the excretion of nitrogen similar to that attributed to the morning meal. The taking of the 300 c.c.

TABLE V.

NITROGEN EXCRETION BY PERIODS.

Subject.	Period.	Jan. 13.	Jan. 14.	Jan. 15.	Jan. 16.	Jan. 17.	Jan. 18.	Jan. 19.	Jan. 20.	Jan. 21.
		grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.
H.	I	1.532	1.392	1.692	1.645	1.983	1.908	1.811	1.551	1.520
	II	1.646	1.568	1.636	1.657	2.826	2.053	1.857	1.679	1.600
	II	1.697	1.560	1.580	1.610	2.956	1.849	1.662	1.762	1.766
	IV	1.965	1.578	1.670	1.530	2.599	1.808	1.638	1.585	1.670
	V	2.072	1.880	1.650	1.718	2.551	1.775	1.589	1.919	2.193
	VI	4.196	4.212	4.766	4.693	6.305	4.967	4.720	4.666	4.910
	Tot.	13.108	12.190	12.994	12.853	19.220	14.360	13.277	13.162	13.659
R.	I	1.890	1.873	1.918	1.643	1.803	2.200	1.847	1.627	1.673
	II	1.835	1.716	2.361	2.134	2.803	2.028	2.124	1.864	2.503
	III	2.134	2.256	2.016	1.785	3.413	1.730	1.919	1.965	2.225
	IV	2.199	1.910	2.334	2.582	3.928	2.912	2.466	2.270	2.115
	V	1.731	1.753	1.670	1.578	2.858	1.975	1.806	1.753	1.182
	VI	4.484	4.691	5.016	4.555	6.653	4.807	4.626	4.926	4.173
	Tot.	14.273	14.199	15.315	14.277	21.458	15.652	14.788	14.405	13.871

of water at 3.30 P. M., followed by the customary ration of milk at supper, making in all very nearly a litre of liquid, may possibly have accelerated the washing out of the urea formed and thus assisted in the attainment of the point of greatest excretion at this hour. These results when considered with the fact that the subjects gen-

[1] Various references cited by SHERMAN and HAWK: *Loc. cit.*

erally did the most fatiguing work of the day between 6.30 P. M. and 9.30 P. M., lead the author to believe that some or all of these factors had at least a slight modifying influence upon the appearance of the high rate of nitrogen excretion at this point.

There was apparently a very well-established tendency to reach the minimum excretion for the day during the long nine-hour night period.

As was to be expected, the taking of the large amount of extra proteid upon January 17 very materially altered the normal curve for the nitrogen excretion. In the first place, instead of showing two well-defined maxima, the excretion of this day showed but one. This maximum occurred with Subject H. during the third three-hour period (12.30 P. M. to 3.30 P. M.) or from six to nine hours after the extra proteid food was ingested. In this respect my results agree with those obtained by Sherman and Hawk.[1] It will be observed that this maximum came at a different hour of the day than either of the daily maxima normally observed preceding the ingestion.

The rise in the rate of excretion after the ingestion of the beef at breakfast was very rapid and began immediately. This sudden rise was followed at once by a fall, somewhat less abrupt than the rise, which continued unchecked even over that portion of the day at which, under normal conditions, the point of most rapid excretion appeared, and extended into the first period of the day following. The most important characteristics of the curves for the two days following the day of the "chief maximum" are the same as those of the curve of the 17th, *i. e.*, one maximum followed by a gradual fall. On the 20th and 21st the normal excretion curve with two maxima (the highest point of excretion being in the fifth period) was again attained. The failure of the excretion of the 17th, 18th, and 19th to show the normal maximum of the fifth period was evidently due in part at least to the fact that a large portion of the extra nitrogen, ingested January 17 at 6.30 A. M., had been excreted before this time of day (6.30 P. M.), and the rate even then being far above the normal, those influences which upon other days were apparently such potent factors in producing a maximum at that point were not able to check this seemingly well-defined tendency of the excretion to reach the normal level of former days.

Taking 12.8 grams as the normal level for the nitrogen excretion

[1] SHERMAN and HAWK: *Loc. cit.*

on the days preceding the beef ingestion, it will be seen that even upon the day of the actual ingestion 6.4 grams or 63 per cent of the 10.1 grams of extra nitrogen was excreted. The total elimination of the extra nitrogen for the forty-eight hours following the ingestion of the beef was 8 grams or 80 per cent, while the last day of the experiment showed 97 per cent of this extra nitrogen eliminated, and the rate of excretion at that time slightly above the level of the normal days mentioned.

Nitrogen excretion by Subject R.— The data for the nitrogen excretion of this subject will be found in Table V, page 128, and the graphic interpretation in Fig. 2.

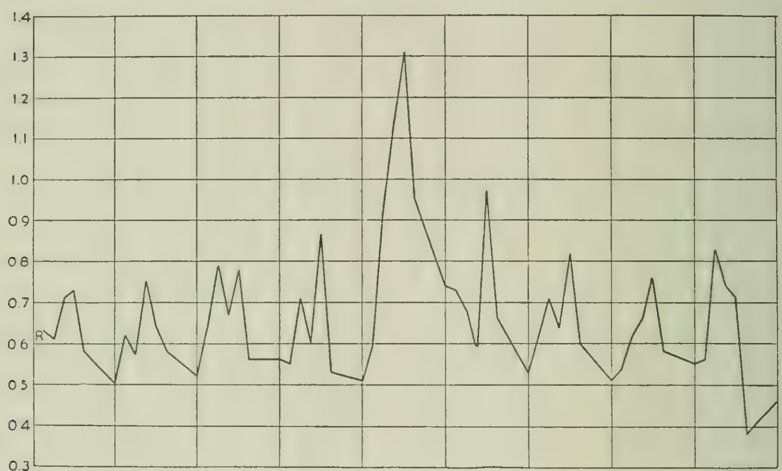

FIGURE 2. — The nitrogen excretion by Subject R. The ordinate shows the average hourly excretion and the abscissa the time.

Subject R. maintained during the preliminary period a normal level very appreciably above the level maintained by Subject H. In general, the curve representing the excretion by Subject R. was similar to that showing the excretion by Subject H., except that the points of maximum excretion were somewhat more accentuated, and also, as has been said, the elimination as a whole was on a higher level. As in the case of Subject H. there was the same very rapid fall in the rate of excretion during the night, and also the accompanying rise. There was a fairly well-defined tendency for the maxima on normal days to occur somewhat earlier than the maxima in the case of Subject H., the point of highest excretion being reached on

normal days generally in the fourth period, instead of the fifth, as with Subject H. By consulting Table IV, page 126, it will be seen that this fourth period was the time of the copious discharge of urine by Subject R., and the author is inclined to think that the passage of such large amounts of water through the system may have tended, in a measure, to cause this occurrence of the maximum excretion at an earlier hour.

Upon the day when the extra proteid was ingested at breakfast, there was the same immediate rise in the rate of excretion as was observed in the graphic representation of the excretion by Subject H. In the present instance, however, instead of reaching its highest point during the third period, as with Subject H., the excretion continued its rapid rise, and only attained its maximum during the fourth period of the day (3.30 P.M. to 6.30 P.M.), nine to twelve hours after the proteid had been ingested, and three hours after the maximum was reached by Subject H. upon the same increased ingestion. Even during the third period, where Subject H. reached the maximum, the excretion of Subject R. was far above that of Subject H., owing to his elimination being at a higher level normally; but when the customary maximum discharge of urine occurred during the next period, it seemed that the excretion by Subject R. followed its normal course and attained its highest point at that time. This fact would seem to indicate, as has already been mentioned, that the large amount of water eliminated during this period had a tendency to give the maximum a somewhat more advanced position than it would have had if the amount of water passed had been no larger than in the contiguous periods. The urea which would have come normally somewhat later, and thus assisted in the formation of the fifth period maximum, shown so plainly in the excretion by Subject H., may, through the agency of this large volume of water, have been removed at an earlier hour, thus causing the second daily maximum for Subject R. to fall in the fourth period.

When we compare Periods I–IV inclusive, of the first four days with the analogous periods of the fifth day, we find that Subject H. eliminated 3.9 grams or 38 per cent of the extra nitrogen during that time, while Subject R. eliminated 3.8 grams or 37 per cent, showing in this respect very marked agreement. Taking into consideration the whole of the day upon which the beef ingestion occurred, we see that Subject R. excreted 7 grams or 70 per cent of the 10.1 grams of extra nitrogen, and showed in this respect an increase of 0.6 grams

or 7 per cent over Subject H. However, during forty-eight hours Subject R. eliminated 8.1 grams or 80 per cent of the extra proteid; this again agreeing with the data for Subject H.'s excretion. During the final day of the experiment Subject R. showed a falling off in the rate of excretion, due no doubt to a storage of nitrogen, and hence no time relation between the consumption of the extra proteid food and the ultimate total elimination of its nitrogen content can be determined.

The days immediately following the day of the "chief maximum" seem to differ from those in the case of Subject H. in the fact that with Subject R. the point of greatest excretion continued to occur at the same hour as on the day of the beef ingestion. This may have been due to the fact that just preceding this period the excretion was at a level, lower in some instances even than the level for the excretion of Subject H. at the same time. Remembering that the normal level for Subject R. was something over one and one-half grams per day above that for Subject H., it is easy to imagine a very natural attempt to at least regain the level, and this attempt being made just at the time that the great surplus of water was eliminated, it is not difficult to see how the maximum may have fallen very naturally at the point indicated.

Sulphur excretion by Subject H. — In general the course of the sulphur excretion followed that of the nitrogen. A notable difference, however, was the greater regularity in the position of the points of maximum excretion in the case of the sulphur. Upon every day of the experiment, including the day when the beef was ingested, there were two well-marked maxima in the excretion of this substance (Fig. 3, page 133). An added regularity was noted in this connection, inasmuch as the first maximum occurred, in every instance, during the third period, while the second maximum fell in the fifth period. Thus on the day of the increased ingestion of proteid following the uniform diet, the "chief maximum" occurred, as in the case of nitrogen, six to nine hours after the beef was eaten.

By reference to Fig. 3, it will be seen that the rate of sulphur excretion was low during the nine-hour night period, but that instead of beginning a sudden rise during the first period of the day following , as was customary with the nitrogen excretion, the course of the sulphur excretion was lower during this period than at any other time in the twenty-four hours. Hence the rise following the ingestion

of the beef began, not immediately after the food had been taken, but only after a lapse of three hours. The rise from this hour was rapid, and the maximum point was reached in the third period. The excretion then returned rapidly to its normal level, which was practically reached by the next morning, and maintained throughout the remainder of the experimental period. In increasing its rate of excretion somewhat tardily after the ingestion of the extra proteid, and in regaining the normal rate at an earlier hour, the excretion of sulphur differed very markedly from that of nitrogen.

As was previously shown by Sherman and Hawk,[1] the ratio between nitrogen and SO_3 was lower on the day following the ingestion of the extra proteid than on any of the other experimental

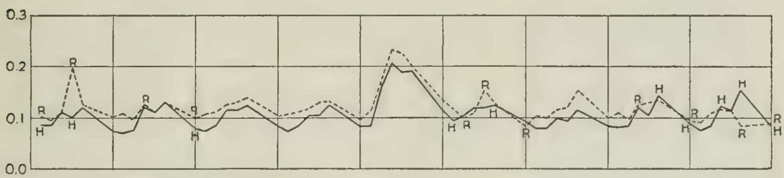

FIGURE 3. — The SO_3 excretion by both subjects. The ordinate shows the average hourly excretion and the abscissa the time. The curve for Subject R. is a broken and that for Subject H. a solid line.

days, due to the fact that the SO_3 reached the normal level more quickly than the nitrogen (see Table VI, page 134).

Sulphur excretion by Subject R. — The curve representing the course of the sulphur excretion of Subject R., while possessing many of the features common to the curve for the excretion of Subject H., differed from the latter in a few notable directions. We fail to find, in our examination of this curve, any such regularity in the occurrence of the maxima as was so plainly exhibited in the excretion of Subject H. Instead of showing two daily maxima, falling in the third and fifth periods respectively, many of the days show but one maximum, and its occurrence seems to be unguided by any well-regulated tendency. There appears, however, to be a somewhat masked tendency toward the formation of maxima in the third and fifth periods, as was so plainly set forth in the graphic representation of the excretion of Subject H. This was shown by the fact that on five days there was a maximum occurring in the fifth period, and on four days in the third period. As was true of Subject H., the rate of excretion

[1] SHERMAN and HAWK: *Loc. cit.*

TABLE VI.

SULPHUR (SO_3) EXCRETION BY PERIODS.

Subject.	Period.	Jan. 13.	Jan. 14.	Jan. 15.	Jan. 16.	Jan. 17.	Jan. 18.	Jan. 19.	Jan. 20.	Jan. 21.
		grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.
H.	I	0.210	0.180	0.195	0.196	0.210	0.239	0.205	0.209	0.198
	II	0.211	0.190	0.208	0.210	0.403	0.258	0.208	0.220	0.215
	III	0.280	0.310	0.288	0.262	0.523	0.305	0.255	0.302	0.318
	IV	0.255	0.285	0.289	0.260	0.475	0.302	0.246	0 266	0.283
	V	0.290	0.325	0.317	0.322	0.480	0.315	0.290	0.358	0.383
	VI	0.590	0.596	0.639	0.645	0.850	0.696	0.655	0.677	0.667
	Total.	1.836	1 886	1.936	1.895	2.941	2.115	1 859	2.032	2.064
	Ratio $\frac{}{N : SO_3}$	100 : 14.0	100 : 15.5	100 : 14.9	100 : 14.8	100 : 15.3	100 : 14.7	100 : 14.0	100 : 15.4	100 : 15.1
R.	I	0.256	0.265	0.262	0.268	0.282	0.292	0.261	0.280	0.233
	II	0.241	0.238	0.278	0.277	0.443	0.249	0.255	0.243	0.270
	III	0.275	0.305	0.313	0.292	0.596	0.281	0.283	0.318	0.306
	IV	0.496	0.280	0.328	0.322	0.573	0.387	0.305	0.325	0.280
	V	0.307	0.321	0.375	0.330	0.498	0.325	0.387	0.332	0.212
	VI	0.761	0.748	0.758	0.720	0.990	0.628	0.732	0.713	0.685
	Total.	2.336	2.157	2.314	2.209	3.382	2.162	2.223	2.211	1.986
	Ratio $\frac{}{N : SO_3}$	100 : 16.4	100 : 15.2	100 : 15.1	100 : 15.5	100 : 15.8	100 : 13.8	100 : 15.0	100 : 15.4	100 : 14.3

during the night was low; but opposed to the conditions which obtained in the excretion of Subject H., the minimum rate of excretion occurred in the nine-hour night period instead of in the first period of the day.

The excretion of sulphur followed that of nitrogen by commencing an immediate rise as soon as breakfast had been taken. Everything considered, there was a fairly satisfactory agreement between the courses of the nitrogen and sulphur excretions of Subject R., though perhaps hardly as close as that shown in the curves for the nitrogen and sulphur elimination of Subject H.

Upon the day when the extra proteid was given, there was the customary rise immediately after the first meal had been taken. This was followed, at the commencement of the second period, by a very pronounced increase in the rate of excretion, which continued until the third period, and formed the "chief maximum" in six to nine hours after the extra proteid was ingested. In this it agrees with the positions of the nitrogen and sulphur maxima for Subject H., but falls three hours sooner than the corresponding maximum for Subject R. As was true in the case of Subject H., the decline from the point of maximum excretion was rapid, and the normal level of the preliminary period was fully regained during the early periods of the day following.

Recalling now the positions occupied by the nitrogen and sulphur maxima of the two subjects upon the day when the added proteid was consumed, we note that three of the four maxima fall in the third period. This may lend some force to the supposition that the maximum for nitrogen in the excretion by Subject R. was carried along to a later period simply through the agency of the unusually large volume of urine passed during that period.

Phosphorus excretion by Subject H. — The course of the phosphorus excretion by Subject H. was somewhat more comparable to the sulphur than to the nitrogen excretion of this subject. We note the same evident tendency toward the formation of two maxima in the third and fifth periods respectively. On some days, however, the highest point was at the end of the night period. This phenomenon formed a very marked contrast with the excretion of nitrogen and sulphur, for in the latter cases the rate of excretion at that time was very low. At the beginning of the first morning period, the rate of the excretion of phosphorus underwent a very rapid fall, and reached a decided minimum at the end of the period. This same character-

istic, in a much less accentuated form, was noted in the sulphur excretion of Subject H. The minimum point in the phosphorus excretion was followed by a rise, just as marked in nature as the fall, and the level of the night period was not regained on normal days, until the excretion had reached the third or fourth period.

On the day of the extra proteid ingestion there was the customary fall immediately after the ingestion, followed by the usual rise. This rise, however, was more prolonged and reached the maximum in the second period, or three to six hours after the meal. The stimulating effects produced by the ingestion of this large amount of proteid food may have been a factor in the formation of this maximum in the second period rather than in the third, as was evidently the course of the phosphorus excretion on the other days of the experiment. Following this "chief maximum," the second maximum occurred as usual in the fifth period, after which the rate descended during the night period and reached the normal level in the first period of the following day.

The constant diet furnished normally 4.96 grams of P_2O_5 (see Table VII, page 137), and upon the day when the extra proteid was ingested this amount was augmented by 0.86 grams. The average total excretion of P_2O_5 for the first five periods of the four days comprising the preliminary interval was 1.47 grams, and the total for the similar periods of the fifth day was 2.09 grams. Thus 0.62 grams, or 72 per cent of the extra P_2O_5 ingested, was eliminated by the urine in fifteen hours after the food was ingested, or in twelve hours after the rate of the P_2O_5 excretion began to rise. In the twenty-four hours following the ingestion of the extra proteid, 0.70 grams, or 81 per cent of the extra P_2O_5 ingested at this time, was eliminated by the urine and the rate of excretion was almost precisely at the normal level maintained during the four preliminary days.

Phosphorus excretion by Subject R. — As was seen to be true of this excretion in the case of Subject H., there was a somewhat closer relationship to the sulphur excretion than to the nitrogen excretion. There was here, however, a very evident inclination toward the formation of but one maximum, which generally fell in the third or fourth period. A falling off in the rate of excretion during the night was noted, and formed in many cases a marked contrast to the rising tendency of the phosphorus elimination of Subject H. The extremely rapid decline in the rate during the first period of the day, which was so plainly set forth in the graphical representation of Subject H.'s

excretion, did not obtain to so great an extent here; but the excretion followed somewhat more closely the moderate course taken by the sulphur excretion of Subject H.

The point of mininum excretion occurred, as with Subject H., during the first morning period, and the failure of the excretion to exhibit the extremely rapid fall so characteristic of the phosphorus

TABLE VII.

Phosphorus (P_2O_5) Excretion by Periods.

Subject.	Period.	Jan. 13.	Jan. 14.	Jan. 15.	Jan. 16.	Jan. 17.	Jan. 18.	Jan. 19.	Jan. 20.	Jan. 21.
		grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.
H.	I	0.164	0.148	0.116	0.123	0.212	0.116	0.096	0.093	0.170
	II	0.289	0.192	0.284	0.272	0.489	0.284	0.245	0.270	0.200
	III	0.235	0.375	0.332	0.322	0.481	0.341	0.337	0.354	0.385
	IV	0.417	0.381	0.333	0.293	0.438	0.355	0.255	0.311	0.385
	V	0.458	0.425	0.358	0.360	0.474	0.357	0.411	0.347	0.415
	VI	1.138	1.049	1.107	1.135	1.189	1.082	0.931	1.267	1.271
	Tot.	2.701	2.570	2.530	2.505	3.283	2.535	2.275	2.642	2.826
R.	I	0.289	0.289	0.312	0.290	0.314	0.254	0.234	0 251	0.258
	II	0 311	0.326	0.414	0.368	0.505	0.297	0.286	0.276	0.398
	III	0.380	0.491	0.525	0.491	0.636	0.375	0.427	0.463	0.455
	IV	0.578	0.460	0.561	0.589	0.547	0.479	0.391	0.404	0.362
	V	0.376	0.505	0.498	0.444	0.533	0.395	0.430	0.363	0.277
	VI	0.946	1.095	1.088	0.966	1.081	0.925	0.935	1.147	1.046
	Tot.	2.880	3.166	3.398	3.148	3.616	2.725	2.703	2.904	2.796

excretion of Subject H., was evidently due to the fact that the single maximum in the case of Subject R.'s excretion occurred either at 3.30 P.M. or 6.30 P.M., and was followed by a gradual fall for a period of fifteen to eighteen hours. Under such conditions, the effect of the extreme rapidity of the customary morning fall would be somewhat neutralized by the falling off in the rate at so early an hour. In many instances with Subject H., as we have seen,

the morning fall, following as it did the gradual rise of the previous long nine-hour night period, caused the graphic representation of the course of the excretion at this point to assume a somewhat more striking appearance than would otherwise have obtained.

Following the ingestion of the beef on the fifth day, the excretion rose rapidly after 9.30 A. M., and reached the "chief maximum" in the third period, or six to nine hours after the ingestion. In this it

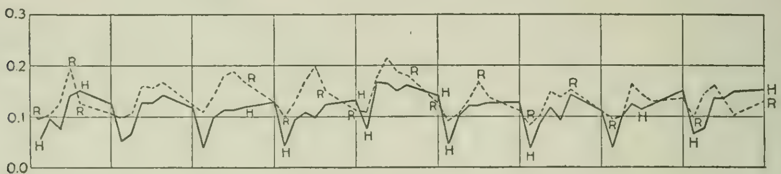

FIGURE 4. — The P_2O_5 excretion by both subjects. The ordinate gives the average hourly excretion and the abscissa the time. The curve for the excretion by Subject R. is in a broken line and that for the excretion by Subject H. in a solid line.

agreed with the excretion of sulphur of both subjects, but occurred three hours later than the "chief maximum" of the phosphorus excretion of Subject H. In descending from this point of maximum excretion there was no formation of a second maximum as occurred in the course of the phosphorus excretion of Subject H. At the end of the first period of the day following the extra proteid ingestion, the level was somewhat lower than the normal level of the preliminary period, and continued practically unchanged to the end of the experiment. But 0.42 grams or 50 per cent of the extra P_2O_5 was excreted during the fifteen hours following the ingestion, and but 0.47 grams or 55 per cent during the whole twenty-four hours, differing in this respect quite materially from the percentage excretion by Subject H. during this time.

Income and outgo of nitrogen (Subject H.). — During the preliminary portion of the experimental period, upon a constant diet affording 14.86 grams of nitrogen per day, Subject H. excreted on an average 12.8 grams, showing an evident storage in the body each day of approximately two grams of the ingested nitrogen. After the extra proteid had been ingested and 80 per cent of its nitrogen content eliminated by the urine inside of forty-eight hours, the rate of excretion of nitrogen again fell, and during the final days of the experiment gave an average daily elimination of 13.37 grams, or about 1.5 grams less than the ingested amount.

Taking into consideration the entire experimental nine days, Subject H. showed a gain of 11.51 grams of nitrogen. We may rightfully assume that the major part of this nitrogen was retained in the tissues of the body. Nevertheless if we apply the figure advanced by Voit[1] for the nitrogen content of fresh muscle (3.4 per cent), it is easily demonstrated that this gain of 11.51 grams of nitrogen, if used entirely for the formation of muscular tissue, could in itself account for more than the total gain of 200 grams body weight. This gain in nitrogen was evidently accompanied by a loss of water from the system, otherwise a greater increase in body weight would appear. The average gain of nitrogen per day was 1.28 grams (see Table VIII).

TABLE VIII.

INCOME AND OUTGO OF NITROGEN (NITROGEN BALANCE).

Subject.	Exp. period.	Length of period.	Nitrogen.				
			In food.	In urine.	In fæces.	Gain or loss.	Average gain or loss per day.
		days.	grams.	grams.	grams.	grams.	grams.
H.	I	4	59.44	51.15	3.02	+5.27	+1.32
	II	1	25.00	19.22	1.42	+4.36	+4.36
	III	4	59.44	54.46	3.10	+1.88	+0.47
	Total.	9	143.88	124.83	7.54	+11.51	+1.28
R.	I	4	59.44	58.06	4.34	−2.96	−0.74
	II	1	25.00	21.46	1.63	+1.91	+1.91
	III	4	59.44	58.71	3.23	−2.50	−0.62
	Total.	9	143.88	138.23	9.20	−3.55	−0.39

The greater amount of nitrogen in the fæces on the day when the extra proteid was taken, over that of the normal days of the milk diet, seems to indicate, either that beef entails a larger amount of metabolic products in its digestion than milk, or, that the normal diet

[1] VOIT: Zeitschrift für Biologie, 1865, i, p. 97.

gave an amount of proteid adequate to the needs of the body, whereas when the extra proteid was taken, the body could not immediately adapt itself to the new conditions, and the nitrogen content of the fæces was consequently raised.

Income and outgo of nitrogen (Subject R.). — Upon an examination of the nitrogen balance for this subject (Table VIII, page 139) we note a marked change in conditions from those which obtained with Subject H. Upon a daily ingestion of 14.86 grams of nitrogen during the preliminary period, there was an average daily elimination of 14.5 grams by the urine. In thus approximating nitrogen equilibrium, the excretion of Subject R. differed decidedly from that of Subject H., which ran on an average level 1.7 grams per day lower. However, during the forty-eight hours following the beef ingestion, 80 per cent of the extra nitrogen was eliminated by this subject, thus showing perfect agreement with the percentage elimination of Subject H. After the effects of the extra proteid had subsided, the excretion again assumed practically the same level it held during the preliminary period. It is thus seen that the normal level for Subject R. during the entire experiment was about 1.7 grams higher than the normal level for Subject H.

As we see from the balance of income and outgo, Subject R. suffered a loss in nitrogen aggregating, during the experiment, 3.55 grams. The loss of 800 grams body weight shown by him at the end of the experiment, may in a measure serve as a means for the explanation of this discrepancy. However, a large part of this 800 grams loss was doubtless due to the abstraction of water from the system. Some time previous to the commencement of this investigation, Subject R. had been upon a diet having a somewhat lower proteid content than that of the constant diet of the experiment. Hence the continuous ingestion of a larger amount of proteid food during the experimental period may have stimulated proteid metabolism and thus served as the chief factor in the production of the loss sustained by this subject.

Income and outgo of sulphur (Subjects H. and R.). — As is universally understood, the method for determining SO_3 in the urine by precipitation as barium sulphate fails to reveal the total SO_3 present. The exact percentage left undetermined is to a certain extent a matter of opinion and speculation. Different writers present different solutions of the problem, and as far as the author is informed, there seems to have been no invariable factor presented as represent-

ing the relation existing between the total SO_3 and that obtained by the precipitation method. It is the author's opinion that no rigid factor can be deduced, inasmuch as it seems to be the tendency of the ratio to vary with the conditions. Circumstances were such during the progress of this investigation as to exclude the determination of the total SO_3 in the urine by the reliable fusion method. Therefore rather than employ any assumed factor in calculating the total

TABLE IX.

Income and Outgo of SO_3 (Sulphur Balance).

Subject.	Period.	Length of period.	Income.	Outgo.		Gain or loss.	Average gain or loss per day.
			In food.	In urine.	In fæces.		
		days.	grams.	grams.	grams.	grams.	grams.
H.	I	4	13.48	7.55	3.74	+2.19	+0.55
	II	1	4.83	2.94	1.11	+0.78	+0.78
	III	4	13.48	8.07	3.84	+1.57	+0.39
	Total	9	31.79	18.56	8.69	+4.54	+0.50
R.	I	4	13.48	9.02	2.25	+2.21	+0.55
	II	1	4.83	3.38	1.19	+0.26	+0.26
	III	4	13.48	8.58	3.48	+1.42	+0.38
	Total	9	31.79	20.98	6.92	+3.89	+0.43

SO_3 from the SO_3 as determined, this somewhat low value for the SO_3 content of the urine was used in the calculation of the income and outgo of sulphur (Table IX). Recognizing the deficiency in the outgo however, all discussion of income and outgo of sulphur has been omitted.

Income and outgo of phosphorus (Subjects H. and R.). — The method for the determination of P_2O_5 in the urine by titration with uranium acetate having been shown in this laboratory[1] to give results closely comparable with those obtained by the fusion method, the author felt

[1] Sherman, H. C.: Bulletin 121, Office of Experiment Stations, United States Department of Agriculture.

he could safely rely upon the data thus secured in the preparation of a phosphorus balance. The striking point about this balance is the large percentage of P_2O_5 eliminated by the fæces (Table II, page 123). In the case of Subject H. for instance, 18.94 grams or 44.2 per cent of the total P_2O_5 elimination passed out through the intestines, and with Subject R. the amount thus passed was the slightly lower value of 17.11 grams or 38.5 per cent of the total elimination.

During the nine days of the experiment, Subject H. gained 2.69 grams of P_2O_5, whereas Subject R. gained but 0.90. This was a

TABLE X.

INCOME AND OUTGO OF P_2O_5 (PHOSPHORUS BALANCE).

Subject.	Period.	Length of period.	Income.	Outgo.		Gain or loss.	Average gain or loss per day.
			In food.	In urine.	In fæces.		
		days.	grams.	grams.	grams.	grams.	grams.
H.	I	4	19.84	10.31	8.27	+1.26	+0.32
	II	1	5.82	3.28	3.01	−0.47	−0.47
	III	4	19.84	10.28	7.66	+1.90	+0.47
	Total	9	45.50	23.87	18.94	+2.69	+0.30
R.	I	4	19.84	12.60	6.10	+1.14	+0.29
	II	1	5.82	3.62	3.45	−1.25	−1.25
	III	4	19.84	11.13	7.56	+1.15	+0.29
	Total	9	45.50	27.35	17.11	+0.90	+0.10

daily gain of 0.3 grams by Subject H., and of 0.1 gram by Subject R. (see Table X). There is thus one point of similarity between the nitrogen balance and the phosphorus balance, in that in each case the gain by Subject H. was far above the gain by Subject R. With the sulphur balance (Table IX, page 141), the conditions were practically identical in the two cases.

Relation between the nitrogen content of the urine and the heat of combustion of its water-free substance.—It has been assumed from work done in this laboratory and elsewhere, that the ratio between the heat of conbustion of urine and its nitrogen content will remain

fairly constant so long as a definite amount of some specific proteid is daily ingested. The previous work seems to indicate that the ratio will be somewhat higher upon a diet of crackers and milk than upon a diet consisting principally of beef. The data from this investigation verify this conclusion. Upon a diet of crackers and milk

TABLE XI.

RELATION BETWEEN THE NITROGEN AND HEAT OF COMBUSTION OF URINE.

Subject.	Date (1900).	Weight of urine.	Heat of combustion per gram.	Total heat of combustion of urine.	Nitrogen excreted.	Ratio of nitrogen to calories.	Calories in excess of urea.[2]
		grams.	small calories.	large calories.	grams.		
H.[1]	Jan. 13	1414.2	74.3	105.1	13.040	1 : 8.1	34.3
	" 14	669.2	157.2	105.2	12.250	1 : 8.4	38.7
	" 15	726.6	153.3	111.4	13.007	1 : 8.6	40.8
	" 16	702.2	151.5	106.4	12.780	1 : 8.3	37.0
	" 17	1080.3	139.0	150.1	19.120	1 : 7.85	46.3
	" 18	740.3	160.1	118.5	14.435	1 : 8.2	40.1
	" 19	723.4	159.5	115.4	13.100	1 : 8.8	44.3
	" 20	768.6	167.8	129.0	13.260	1 : 9.7[3]	57.0
	" 21	680.6	170.4	116.0	13.480	1 : 8.6	42.8

[1] The urine of Subject R. decomposed before satisfactory analyses could be made.

[2] See SHERMAN and HAWK: *Loc. cit.*

[3] Paraffin was used for sealing sample bottles and a few small pieces were found in this composite urine sample. The heat of combustion of paraffin being about 9075 small calories per gram, the combustion of a very minute portion could easily cause the variation in the ratio noted on this day (20th).

during the four preliminary days, an average ratio of 1 : 8.3 was maintained. Upon the fifth day, when the constant diet was altered so as to secure a change in the ingestion from milk proteids to beef proteids, the ratio fell immediately to 1 : 7.85, but regained its former level when the constant diet of crackers and milk was again taken upon the following days.

As was shown by Sherman and Hawk[1] the "calories in excess of urea" assume a fairly constant level for the whole experimental

[1] SHERMAN and HAWK: *Loc. cit.*

period, and are to a great extent unaffected by the variations in the excretion of nitrogen. This seems to indicate that the organic matter less highly oxidized than urea was eliminated in fairly constant amounts from day to day.

Conclusions.

The normal curve for the nitrogen excretion by each subject showed two points of maximum excretion. On the day of the extra proteid ingestion a single maximum was observed, and the return to the normal condition of two maxima occurred on the second day following with Subject R. and on the third day following with Subject H.

After the ingestion of the extra proteid, the nitrogen excretion began an immediate, rapid rise to the point of maximum excretion, which was reached in six to nine hours with Subject H., and in nine to twelve hours with Subject R. This point of maximum excretion was followed at once by a very rapid fall which passed, in a few hours, into a more gradual return to the normal rate of excretion.

The minimum rate of nitrogen excretion for each subject occurred during the night period.

In general the course of the sulphur excretion followed that of the nitrogen. With Subject H. the sulphur excretion of every day of the experiment showed two well-defined maxima; the excretion by Subject R. did not show this regularity.

The sulphur excretion of Subject H. was lowest during the first period of the day; the corresponding excretion of Subject R. was lowest during the night.

After the ingestion of the extra proteid, the sulphur excretion by Subject H. began to rise after the lapse of three hours; the sulphur excretion by Subject R. began to rise at once.

The maximum sulphur excretion occurred with each subject six to nine hours after the ingestion of the extra proteid. The normal rate of excretion was regained in twenty-four hours.

The course of the phosphorus excretion by Subject H. showed a tendency toward the formation of two maxima daily; the phosphorus excretion by Subject R. generally showed a single maximum.

The minimum phosphorus excretion by each subject occurred during the first three-hour morning period.

After the ingestion of the extra proteid at breakfast, the customary

fall in the phosphorus occurred, and was followed by a rise which caused the excretion to reach the maximum in three to six hours after the ingestion with Subject H., and six to nine hours after the ingestion with Subject R. The normal level was regained with each subject at the first period of the following day.

The ratio of the nitrogen content of the urine to the heat of combustion of its unoxidized material was somewhat lower on the day of the extra proteid ingestion than on normal days.

The ratio between nitrogen and SO_3 was the lowest on the day following the ingestion of the extra proteid, due to the fact that the SO_3 reached the normal level more quickly than the nitrogen.

On a constant diet accompanied by like water ingestion, one subject excreted but 60 per cent as much urine as the other.

The author wishes to express his gratitude to Prof. W. O. Atwater and Dr. H. C. Sherman for many valuable suggestions and for their continued interest in the investigation. His thanks are also due Mr. A. E. Roberts, who acted as subject and assisted in the analytical work, and also Messrs. E. Osterberg, W. R. Frazier, and E. M. Swett, who were very helpful in carrying out the details. The author is also very grateful to Mr. R. D. Milner for many suggestions during the preparation of this paper.

ON THE DISTRIBUTION OF OSSEOMUCOID.

By CHRISTIAN SEIFERT and WILLIAM J. GIES.

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THE question whether or not bone contains glucoproteid seemed to be settled in the negative in 1892, when Young, working under Halliburton's superintendence, failed to extract from bone, with calcium hydroxide or barium hydroxide, any substance that could be precipitated with acetic acid.[1] Several years ago, however, on investigating this matter, we were able to show in this laboratory that the rib and femur of the ox contain an appreciable quantity of mucoid.

In our first communications [2] on this subject we outlined the method of separating this substance, which has been termed osseomucoid, and we also pointed out the mechanical obstacles in the way of success in the method employed by Young for its detection. Later, our studies in this connection were devoted to perfecting the method of separating osseomucoid, and to determining its composition, reactions and heat of combustion.[3]

On failing to detect mucoid in the bone shavings and powder under examination, Young concluded that "in the process of ossification the connective tissue matrix is apparently completely calcified." The results of our own work proved, however, that in the ossification of at least the femur and rib of the ox, the "connective tissue matrix" is *not* entirely removed. Until osseomucoid could be shown to exist in

[1] YOUNG: Journal of physiology, 1892, xiii, p. 213.

[2] GIES: Proceedings of the American Physiological Society, This journal, 1900, iii, p. vii; Proceedings of the American Association for the Advancement of Science, 1900, p. 131; Biochemical Researches, 1903, i (a and b), pp. 31–33.

[3] HAWK and GIES: This journal, 1901, v, p. 387; MEAD and GIES: Proceedings of the American Physiological Society, This journal, 1902, vi, p. xxviii; GIES: Biochemical Researches, 1903, i (bb), p. 53, and reprints Nos. 2 and 3.

the bones of other animals it was impossible, of course, to say that Young's deduction as to complete substitution of the "connective tissue matrix" during ossification, was not, perhaps, correct in the main.

During the past two years we have been making a study of the distribution of osseomucoid in the bones of various animals.[1] Our purpose thus far has been to ascertain merely the extent of its distribution in animal species rather than in the various bones of individuals. Our work in this connection has been wholly qualitative.

The bones under examination were, in practically all cases, the larger osseous structures of the limbs. They were thoroughly freed of all extraneous matter and subjected to the treatment in acid described for previous preparations.[2] Large bones were converted into shavings after treatment with dilute hydrochloric acid. Small bones were merely softened in the dilute acid and when nearly all of the inorganic matter was removed they were thoroughly minced in a meat-chopper. The quantities of moist ossein in each experiment varied from a few grams to several hundred, the amount in use depending on the bulk of the available bony material. The lime water extracts were treated with acid in excess, as usual, and the precipitates were thoroughly tested to determine mucoid identity.

The results of our chemical examinations of the precipitates were invariably as follows:

A. After thoroughly washing free from acid, each product thus obtained was found to be acid to litmus.

B. Each precipitate gave proteid color-reactions.

C. Each substance was free of phosphorus.

D. On hydration in pure hydrochloric acid, solutions of each product were formed which always contained:

- *a*. An insoluble proteid portion.
- *b*. Proteose and peptone.
- *c*. Sulphate.
- *d*. Reducing substance.

E. Each precipitate readily dissolved in dilute solutions of alkalies and of alkaline salts.

F. Each substance was insoluble in a moderate excess of cold mineral acid, as well as organic acid.

[1] GIES: Proceedings of the American Physiological Society, This journal, 1903, viii, p. xiii; Biochemical Researches, 1903, i (cc), p. 54.

[2] GIES: *Loc. cit.*

These results made it evident that all of our precipitates consisted of mucoid.

By these methods, osseomucoid was detected in, and separated from, the larger bones of each of the following animals:[1]

Mammals.	*Birds.*	*Reptiles.*	*Fish.*
Man	Woodpecker	Alligator	Cod[2]
Rabbit	Sea gull	Turtle	
Seal	Partridge		
Cat	Chicken		
Dog	Turkey		
Black bear	Marsh hawk		
Ox	Blue heron		
Sheep	Surf snipe		
Deer	Flamingo		
Caribou	Ostrich		
Pig			
Tapir			
Kangaroo			

Our results make it seem very probable that osseomucoid is a normal constituent of *all* bones.

We take pleasure in acknowledging our obligations to Prof. Henry F. Osborn, and to Director Wm. T. Hornaday of the New York Zoological Park, for their interest in this research and for much of the material under investigation. We are also indebted to Mr. Alfred Malik for assistance in procuring some of the animals.

[1] All bones tested thus far have yielded positive results.

[2] The bones of the head were the ones examined.

ON THE VARIATIONS OF BLOOD-PRESSURE DURING THE BREATHING OF RAREFIED AIR.

By FREDERIC H. BARTLETT.

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DURING the summer of 1903 it was my privilege to carry out the following investigation under the direction of Professor Kronecker. I wish to take this occasion of expressing my gratitude to him for his constant suggestion and unflagging interest in the work. Indeed the whole investigation belongs to him in its origin and plan.

The following study is one of a series which Professor Kronecker has made in the course of his inquiry into the causes of mountain sickness, and its results have contributed further confirmatory evidence of his theory as stated in his "Gutachten über die Frage: Ob und unter welchen Bedingungen sowohl der Bau als der Betrieb einer Eisenbahn auf die Jungfrau ohne ausnahmweise Gefährdung von Menschenleben (Gesundheit) möglich sei."[1] He there maintains that mountain sickness is caused by disturbances of the circulation, saying that "the pulmonary vessels, exposed to the diminished air-pressure found also in the lungs, dilate and thereby produce congestion in the lesser circulation."[2]

[1] KRONECKER: Beilagen zum Konsessions-Gesuch für eine Jungfraubahn, Zurich, 1894.

[2] KRONECKER: *Ibid.*, p. 47. "Wohl derart, dass unter dem auch in der Lunge verminderten Luftdruck die Blutgefässe der Lunge aufschwellen und hierdurch Stauungen in kleinen Kreislaufe entstehen."

The views of Albrecht von Haller, the early Bernese investigator, are interesting in this connection, as he also sought for an explanation of mountain sickness in circulatory disturbances. Rarefied air, he maintained, is injurious because it does not fully expand the lungs. Pressure is withdrawn from the vessels of the whole body, which therefore offer less resistance to the heart and are easily ruptured. Since denser air expands the lungs better, facilitating the passage of blood through them, better supplying the left heart with blood, and making it contract more strongly, we can with difficulty bear sudden changes in air-pressure such as we have in high altitudes. Rarefied air expands the lungs incompletely and thus withdraws the stimulus that excites the left heart to contraction. Elementa Physiologiae Corporis Humanii, iii, pp. 194–197.

I tested this theory by making rabbits breathe from a bottle constantly supplied with rarefied air from Waldenberg's apparatus.[1] This is constructed on the same principle as the gasometers in gas factories. Its outer cylinder, 1 metre high and 30 centimetres in diameter, contains water. The escape tubes being closed, the density of the air is regulated by pressing down or drawing up the inner cylinder by means of weights. The bottle from which the rabbit breathed contained five litres, and was connected by a rubber tube with the escape-tube of the cylinder. Two other tubes entered the bottle through the same cork as the first. One opened into the outside air and constantly supplied the bottle with from one and a half to five litres of fresh air a minute, about five times as much as the rabbit actually needed; the other ended in a quicksilver manometer for measuring the air-pressure in the bottle. The rabbit breathed through a tracheal cannula connected with a short tube which entered the bottle near the bottom. The corks and tubes were sealed with paraffin. The rabbit was first given an injection of morphine sufficiently large to prevent pain, the reflex sensibility, however, being increased. The blood-pressure was measured by means of a manometer connected with the common carotid artery, and arranged to write on a Ludwig Kymograph.

The tables which follow will show in what way the blood-pressure and respiration changed with the variations in the pressure of the air.

[1] WALDENBERG: Centralblatt für die medicinischen Wissenschaften, 1847, p. 106.

TABLE I. July 14, 1903.

Duration of breathing.	Difference of air-pressure.	Blood-pressure.	Pulse frequency.	Respiratory frequency.	Respiratory excursion.	Remarks.
min.	mm. Hg	mm. Hg				
3	0.0	90	160	36	10	
8	−3.6	88	160	38	8	Several convulsive movements.
11	0.0	90	160	38	8	
10	−5.1	84	164	62	6–8	Noise in room — convulsive movements. 3½ litres fresh air per min.
4	0.0	86	160	44	7	
6	−7.0	82	164	62	10	
10	0.0	85	158	34	8	Noise in room — convulsive movements.
9½	−8 1	81	130	68	4–8	Frequent convulsive movements at end of period. 2 litres fresh air per minute.
2	0.0	90	156	36	6	
5	0.0	88	....	....	8	
6	−8.8	86	....	....	8–10	3 strong convulsive movements at equal intervals during period.
9	0.0	90	....	....	....	Constant convulsive movements, result of noise in room and handling. Blood-pressure at close, 90.
5	−9.6	82	168	70	4	
1½	0.0	92	....	....	8	
10	−10.4	86	....	....	5	Dyspnœa at close of period; four convulsive movements at equal intervals during period.
13	0.0	94	....	....	4	Several convulsive movements.
12	−13.7	88	180	80	4–8	Several convulsive movements.
1¾	0.0	101	....	....	4	

Rabbit extremely irritable throughout experiment.

TABLE II. July 15, 1903.

Duration of breathing.	Difference of air-pressure.	Blood-pressure.	Pulse frequency.	Respiratory frequency.	Respiratory excursion.	Remarks.
min.	mm. Hg	mm. Hg				
5	0.0	106	260	16	3	In this experiment a water manometer was used, in others a mercury manometer.
3	0.0	108	....	....	4–8	Rabbit breathed air from flask with no ventilation. No sign of dyspnœa. Two convulsive movements.
15	--5.5	104	250	26	2	4 litres fresh air per minute.
6	0.0	105	220	16	3	Several convulsive movements.
7	−6.6	104	240	26	3	Several convulsive movements.
4	0.0	104	240	18	6	Noise in room caused several convulsive movements.
6	−8.1	102	275	28	4	Several convulsive movements. 4 litres fresh air per minute.
5	0.0	104	260	20	8	Several convulsive movements.
7	−9.6	100	320	30	3	Several convulsive movements.
4½	0.0	108	....	....	6	Writing on fresh paper. Several convulsive movements.
6½	−11.8	108	....	....	4	
7	0.0	109	270	24	4	
7½	−17.0	106	320	110	....	Expiration very active.

TABLE III. July 17, 1903.

Duration of breathing. A. M.	Difference of air-pressure.	Blood-pressure.	Pulse frequency.	Respiratory frequency.	Respiratory excursion.	Remarks
min. sec.	mm. Hg	mm. Hg				
15 15	0.0	122	....	....	2	
37 20	−20.0	118	208	128	2–4	Experiment to test ventilation of flask. Except for slightly forced expiration, no dyspnœa. Three or four convulsive movements.
60	0.0	....	188	52	....	
35	−20.0	116	180	150	2–4	No dyspnœa.

Same rabbit. In the afternoon, −20 caused slight dyspnœa but no asphyxia. After cutting vagus at −20, marked dyspnœa appeared, which was relieved by fresh air. The blood-pressure thereupon fell. At −25 asphyxia ensued in a few seconds.

TABLE IV. July 21, 1903.

Duration of breathing.	Difference of air-pressure.	Blood-pressure.	Pulse frequency.	Respiratory frequency.	Respiratory excursion.	Remarks.
min. sec.	mm. Hg	mm. Hg				
6 50	0	136	....	....	12–22	Blood-pressure and respiratory excursion very irregular.
5 32	0	132	360	26	10–22	During this period rabbit breathed out of flask with no ventilation. No dyspnœa.
8 25	−3	123	340	30	8	Rabbit moved two or three times in middle of this period. Respiratory excursion, 30.
5 30	−20	116–90	....	76	5	In middle of period blood-pressure fell to 90, and then immediately rose again to 104.
0 50	−20	....	....	....	28	Several convulsive movements, but no indication of dyspnœa.
13 40	−3	122	200	26	6	One convulsive movement.
4 5	−20	106–92	....	....	2	Blood-pressure fell steadily to 92.
2 57	−20	....	....	....	14–30	Series of convulsive movements.
3 12	−3	106–116	....	....	3	Steady rise of pressure.
4 57	−25	90.106	....	....	2	Blood-pressure varied greatly. Figures represent maximum and minimum. Minimum occurred in middle of period.
2 55	−25	98–112	120	120	4–8	Dyspnœa throughout, with constant rise of pressure. Vagus irritation at close.
4 5	−3	90.104	....	....	2–10	Breathing and blood-pressure both irregular.
3 10	−20	88.101	....	....	1	At start, blood-pressure fell immediately to 88, then rose to 100, and again fell steadily to 88 at close.
1 25	0	90–100	....	....	2	
0 45	−25	89	....	...	1	Blood-pressure rose to 106 and then fell to 89.
1 25	−25	....	....	....	....	Dyspnœa at outset, followed by asphyxia and vagus irritation. Blood-pressure showed characteristic rise.
2 55	−3	102				
1 25	−3	68–92	....	....	....	Cut vagi at beginning of period. Blood-pressure fell immediately to 68 and rose gradually to 92.
1 10	−20	82–116	....	....	4–8	Blood-pressure rose rapidly to 116. Marked dyspnœa.
1 12	−13	124	....	....	12	Continued dyspnœa.
4 15	0	122	....	....	24	Continued dyspnœa. Tried artificial respiration, but could not restore normal pressure and respiration

TABLE V. July 22, 1903.

Duration of breathing.		Difference of air-pressure.	Blood-pressure.	Pulse frequency.	Respiratory frequency.	Respiratory excursion.	Remarks.
min.	sec.	mm. Hg	mm. Hg				
3	55	0	96	195	16	5	
4	30	−2	98	....	....	6	
2	30	−20	92	212	50	2	
3	20	−20	94	....	....	4	Dyspnœa throughout.
3	35	−2	86	220	28	3	
2	10	−15	86	194	84	2	
1	37	−15	88	....	....	3	Dyspnœa throughout. Forced expiration.
3	55	0	85	210	24	3	
7	0	−3	96	....	....	6	
1	45	−20	95–100	....	....	8	Dyspnœa set in immediately at beginning of period.
1	35	−2	94	....	....	6	
2	25	−2	99–94	....	....	14–8	Rabbit handled at beginning of period.
1	45	−10	96	....	....	5	Breathing difficult, sterterous sound.
1	0	0	93	....	....	6	Cut vagi immediately after this period.

Note extreme susceptibility to change of pressure shown by this rabbit. Autopsy showed no particular lung lesion.

TABLE VI. July 23, 1903.

Duration of breathing.		Difference of air-pressure.	Blood-pressure.	Pulse frequency.	Respiratory frequency.	Respiratory excursion.	Remarks.
min.	sec.	mm. Hg	mm. Hg				
7	50	0	97	180	28	2	
6	15	–3	93	162	24	2	
3	12	–20	88	123	48	6	
4	45	–3	92	156	28	2	
7	52	–14	83	....	40	4	Forced expiration.
4	14	0	89	156	20	2	
4	14	–2	88	144	24	3	
2	2	–14	86	....	....	4	
2	56	–18	84	136	39	5	Forced expiration. Noise at end caused convulsive movements with rise of blood-pressure.
4	14	–2	89	....	....	3	Several convulsive movements.
9	55	–14	84	132	38	4	
3	4	–3	86	152	24	2	
4	5	–10	84	156	32	2	
4	23	–13	82	151	36	3	Forced expiration
3	21	–18	83	....	....	4	
2	46	–18	84	....	....	4	Period began with strong convulsion with marked rise of blood-pressure.
3	12	–18	90	....	....	2	Period began with strong convulsion.
3	56	–23	79	....	....	4	Slight nose movement throughout.
3	21	0	92	....	....	1	Period began with convulsion.
2	2	0	108	....	....	6	Fresh drum. Rabbit disturbed in change of drum.
0	52	–29	110	....	....	6	Slight dyspnœa.
1	30	–29	116	....	....	8	Dyspnœa throughout.
1	10	–20	114	....	....	6	Less dyspnœa. Gradual rise of blood-pressure to 120 at close.
1	27	–20	112	....	....	24	Dyspnœa markedly less; vagus irritation.
2	10	0	104	....	....	1½	
3	11	–3	106	....	....	2	
2	55	–20	100	....	....	3	Noise in room raised pressure. Fell to 101 at close.

TABLE VI — (*Continued*).

Duration of breathing.	Difference of air-pressure.	Blood-pressure.	Pulse frequency.	Respiratory frequency.	Respiratory excursion.	Remarks.
min. sec.	mm. Hg	mm. Hg				
2 37	–23	100	....	....	6	Gradual rise in pressure till end of period at 107.
2 45	–14	99	....	....	3	This period began at 109 blood-pressure and followed a period of marked vagus irritation at –20. Reading for –20 imperfect, so omitted.
1 25	–2	104	....	....	2–4	Respiration irregular; slight nose movement.
1 25	–14	105	....	....	$1\frac{1}{2}$	Spasm after 30 seconds, with fall of pressure.
1 20	0	107	....	....	1	Spasm after 35 seconds, with fall of pressure.
2 30	–14	112	....	....	2	Convulsion at close, with fall of pressure.
2 45	–10	112	....	....	2	One convulsion in middle of period and several at close, with fall of pressure.
1 35	0	122	....	....	$1\frac{1}{2}$	Convulsions at close of period, with fall of pressure.
1 45	–20	116	....	....	4	
1 25	–20	103	....	....	14	Very slight nose movement, marked vagus irritation.
3 12	–14	108	....	....	2	Just before close of period, convulsions. At close vagi divided, followed by dyspnœa and immediate rise of pressure.
1 27	0	130	....	....	30	Uniform vagus irritation throughout period.
2 45	0	120	....	....	6	
2 50	–10	130	....	....	6	Marked nose movements.
1 0	–10	130	....	....	14	Vagus irritation.
4 40	0	130	....	....	6	In middle of period, vagus irritation for 35 seconds.

TABLE VII. July 24, 1903.

Duration of breathing.		Difference of air-pressure.	Blood-pressure.	Pulse frequency.	Respiratory frequency.	Respiratory excursion.	Remarks.
min.	sec.	mm. Hg	mm. Hg				
1	55	0	104	....	....	4	
4	21	–3	104	180	18	6	$1\frac{1}{8}$ litres fresh air per minute.
2	0	–20	100–92	....	....	5	
0	25	–20	....	....	....	10–30	Movement of rabbit on being handled.
7	15	–20	92	152	30	4	
0	54	–3	100				
1	30	0	....	....	....	10–30	Several spasms. Rabbit in bad position.
1	55	–3	104	180	24	5	
1	55	–24	98	160	42	8	Slight dyspnœa.
1	45	–20	94	....	....	....	Less dyspnœa than at — 24.
1	55	–3	98				
0	30	–20	93	....	....	4	
1	45	–20	....	....	....	10–20	Dyspnœa and spasms.
4	5	0	120	....	....	....	High pressure from spasmodic movements.
1	20	–3	102	164	24	7	$1\frac{1}{2}$ litres of air per minute.
3	15	–20	92	162	36	6	Expiration forced. Slight nose movements.
1	55	–22	90	152	40	7	Dyspnœa, with rise of pressure in last ten seconds.
2	0	–20	92	....	....	4	
0	35	–3	100	....	....	6	Spasm at end.
0	50	0	116	....	....	8	
0	52	0	116	....	....	12	Several spasmodic movements.
2	20	0	100	....	....	6	Blood-pressure taken at end of period.
0	39	–3	100	....	....	5	
1	27	–20	92	180	36	4	
1	45	–3	100	....	....	8	
1	35	–14	....	....	....	8–30	Continued spasms.

TABLE VII. — (*Continued.*)

Duration of breathing.		Difference of air-pressure.	Blood-pressure.	Pulse frequency.	Respiratory frequency.	Respiratory excursion.	Remarks.
min.	sec.	mm. Hg	mm. Hg				
6	40	0	110	....	....	8	
6	45	−20	107–96	164	105	4–6	Breathing irregular. Expiration explosive. One spasm. Slight nose movement.
2	20	−3	100	....	....	6	One spasmodic movement.
3	10	−14	96	175	....	5	
2	30	−20	92	....	....	4	
1	25	0	100	....	....	7	
1	10	−22	94	....	....	6	
1	10	0	98	....	....	4	
1	5	−3	99	....	....	2	
1	30	−20	94	....	....	3	
3	15	−29	89	126	156	4	Slight nose movements.
3	15	−29	90–98	....	....	6–10	Increasing dyspnœa Active nose movements.
0	37	−29	....	....	....	18–40	Asphyxia.
2	5	0	98	....	....	....	
2	0	0	110–104	....	....	....	Vagi cut at end of last period. Spasmodic movements continue. Blood-pressure high.
5	10	−20	....	....	....	....	Gradual rise of blood-pressure, increasing dyspnœa, and finally convulsions.

TABLE VIII. July 27, 1903.

Duration of breathing.	Difference of air-pressure.	Blood-pressure.	Pulse frequency.	Respiratory frequency.	Respiratory excursion.	Remarks.
min. sec.	mm. Hg	mm. Hg				
1 45	0	96	....	....	6	
3 50	–3	94	180	28	6	
2 55	–3	....	....	....	14–30	Noise in room caused spasmodic movements of rabbit. Cannula final separated from flask.
6 45	–3	97	....	....	5	Quiet breathing, undisturbed.
8 10	–20	82	176	54	5	
1 30	–3	....	....	....	8–30	Rabbit moved several times. Blood-pressure much raised.
1 50	0	....	....	....	12–30	Noise caused movement. Blood-pressure much raised.
4 20	0	92	206	16	7	
4 20	0	....	....	....	6–25	Rabbit moved several times; extremely irritable.
1 45	–3	....	....	....	6–22	Moved again.
4 20	–3	96	214	28	6	
1 30	–3	....	....	....	10–30	Noise in room. Several movements.
3 37	–3	94				
1 25	–3	....	....	....	10–30	Spasm,—rabbit handled.
2 45	–3	93	....	....	5	
4 40	–20	85	160	50	8	At start blood-pressure rose, but fell immediately.
1 30	–3	....	....	....	4–24	Continual movements.
2 40	0	97	....	....	6	Rabbit moved once; duration of effect 15 secs. Blood-pressure and respiratory excursion are for period of quiet.
1 45	0	96	....	....	6	
1 35	–3	....	240	28	5	Rabbit moved at very beginning.
1 45	–10	93	240	....	3	
50	–10	....	....	....	14	Movement, due to noise in room.
3 10	–13	....	204	40	4	
6 40	–3	94	....	....	6	
6 40	–20	82	150	52	8	Nose movement became manifest and expiration forced.
3 30	–28	80	....	....	12–50	After preliminary fall of pressure, dyspnœa and rise of pressure began at once. In 1 min. 15 secs. spasms began; 45 secs. later continued convulsions.

TABLE IX. July 30, 1903.

Duration of breathing.	Difference of air-pressure.	Blood-pressure.	Pulse frequency.	Respiratory frequency.	Respiratory excursion.	Remarks.
min. sec.	mm. Hg	mm. Hg				
5 0	0	110	168	14	8	
0 45	−20	102	....	....	4	
1 10	−20	102	....	36	12	Dyspnœa.
2 0	−3	114	....	....	5	
0 30	−13	104	....	....	7	
1 35	−13	108	....	34	18	Dyspnœa.
0 20	−13	....	....	....	44	Asphyxia. Vagus irritation.
1 40	0	101	....	26	5	Marked depression of blood-pressure after asphyxia.
4 30	0	108	206	16	5	
5 0	−3	104	204	23	8	
0 45	−10	102	....	....	8	
1 25	−10	....	....	....	24	Dyspnœa followed by asphyxia.
0 3	0	100	....	....	2	Marked depression of blood-pressure after asphyxia.
1 5	0	102	184	....	2	
5 25	0	102	....	....	2	
1 10	−3	100	....	....	3	
1 55	−8	99	212	60	3	
2 30	−9	98	240	80	2	
2 25	−10	98	256	84	2	
2 25	−11	97	316	120		
4 45	−13	96	222	150	2	After 2 min. 30 secs. convulsive movement — respiratory excursion 40 — after which blood-pressure fell to 93 to end of period.
2 45	−17	95	192	160	2	
4 20	0	102	222	....	4	At end of period a delay of several minutes to remove blood clot in cannula.
12 0	0	107	....	....	4	At close of period blood-pressure 100, the average being about 107.
1 45	−3	99	184	....	4	
5 15	−20	91	156	176	3	
0 40	−20	90	....	....	10	Dyspnœa throughout. Blood-pressure 95 at close.
4 5	0	98	....	....	4	
2 0	−10	94	....	160		
0 33	−3	96				
1 45	−20	92	....	....	....	Dyspnœa in 15 secs., followed in a few seconds more by asphyxia.

The following figures are taken from the above tables to illustrate more forcibly the relation between changes in air-pressure and changes in blood-pressure.

Diff. of air-pressure.
0, –20, 0, –20, 0, –20, 0, –20, 0, –28, 0, –20, 0, –20, 0, –20, 0, –20.
Blood-pressure.
110, 102, 102, 90, 96, 82, 92, 85, 97, 80, 104, 92, 110, 92, 97, 88, 136, 90.
Diff. of air-pressure. –3, –20.
Blood-pressure. 122, 92.

These tables show clearly the general fact that rabbits react to slight variations in atmospheric pressure. A rarefaction of the air corresponding to 300 metres elevation caused in every case marked difficulty of breathing and in some cases asphyxia.[1] The specific results of the investigation may be stated as follows:

1. Rabbits breathing rarefied air show in the aortic system a fall of blood-pressure. Up to a certain point the fall is greater with the increase of rarefaction, the relation varying with different individuals. The maximum fall of pressure was 46 mm. Hg and occurred with rarefied air of –20.

2. Rapid rarefaction appears to lower the blood-pressure more than does gradual rarefaction.

3. For the most part, rarefaction diminishes blood-pressure suddenly, but sometimes gradually.

4. When the pressure has been reduced from –20 to –32, the animal suffers from dyspnœa and asphyxia, and the blood-pressure rises. Slowing of pulsation by vagus irritation arising from dyspnœa lowers it again: spasms raise it.

5. The pulse frequency shows no clear relation to the rarefaction, but in general decreases with the pressure.

6. The respiratory frequency increases with the rarefaction.

7. The respiratory excursions of the blood-pressure are weaker when the rabbit is breathing rarefied air.

8. Rabbits with cut vagi experience dyspnœa even at normal air-pressure. Their blood-pressure rises with slight rarefaction, and remains high, even if they are allowed to breathe free air again.

The most important of these deductions is the first: the fact that

[1] For example:

–10 mm. Hg = 105 metres rise of altitude.
–20 mm. Hg = 212 metres rise of altitude.
–30 mm. Hg = 321 metres rise of altitude.

the blood-pressure in the aortic system sinks when the pressure of the respired air falls. The point at which the blood-pressure falls varies in each individual case, many rabbits resisting a pressure of −15, others showing a fall in blood-pressure with −10.[1]

The question now arises: What phenomena present themselves in the pulmonary circulation at the same time with the fall of pressure in the aorta system? According to Waldenburg's theory, this fall of pressure is accompanied by a reduction in the capacity of the arteries.[2] The combined result of the diminished pressure and of the reduced arterial capacity must be a swelling of the lung capillaries and a lessening in the outflow of the blood from the lungs. Tigerstedt's recent experiments on rabbits give further proof of this. He has shown that the systolic blood-pressure in the pulmonary artery of the rabbit is equivalent to 11–15–25 mm. of mercury, and that by reducing the atmospheric pressure so that it is equal to the intrapleural pressure hardly any propelling force would remain and the complex symptoms of lung swelling would result.[3] Our experiments have shown that by reducing air-pressure 15–25 mm., a marked fall in blood-pressure is produced. The two sets of figures afford an interesting comparison, and seem clearly to indicate that a reduction of from fifteen to twenty-five millimetres of mercury is enough to bring about that relation between intrapleural pressure and atmospheric pressure which will cause the symptoms of lung swelling and of storing of blood in the pulmonary vessels.

As to the nature of the lesion produced by stagnation of blood in the lungs, I will cite but one reference. The experiments were carried out by Welch and Cohnheim, and consisted in ligaturing the branches of the aorta. They found out that "Passive œdema first occurs in the lungs when the obstruction which hinders the outflow of the blood from the pulmonary veins can no longer be overcome by the action of the right ventricle."[4]

[1] It is clear that animals or men who are wholly within chambers of rarefied air can endure a much lower pressure (400 mm. Hg or even lower) than those who simply breathe rarefied air.

[2] Waldenburg, L.: Die Messung des Pulses und des Blutdrucks am Menschen, Berlin, 1880, pp. 140–141.

[3] Tigerstedt: Skandinavisches Archiv für Physiologie, 1903, xiv, p. 285.

[4] Cohnheim: "Es in dem Lungen erst dann zum Stauungsödem kommt, wenn das Hinderniss, welches dem Abfluss des Blutes aus den Lungenvenen entgegensteht, von der Action des rechten Ventrikels nicht mehr überwunden werden kann." Gesammelte Abhandlungen, Berlin, 1885, p. 594.

We may conclude, then, that there exists in mountain sickness an increased amount of blood in the pulmonary vessels, due to an increase in their capacity and to a stagnation of blood arising from an equalization of the atmospheric and the intrathoracic pressures. The lesions arising from this are comparable to those observed in the experiments of Welch and Cohnheim. The symptoms are analogous to those seen in dyspnœa and asphyxia, both of which invariably appeared in our experiments, the stage of their appearance being different with different rabbits. Finally, the symptoms of mountain sickness are much aggravated in those who, in high altitudes, must work. We found that by cutting the vagus of rabbits the effects of rarefied air are much intensified.

REACTIONS TO TEMPERATURE CHANGES IN SPIRILLUM, HYDRA, AND FRESH-WATER PLANARIANS.

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THE general reactions of Spirillum, Hydra, and fresh-water Planarians has recently been worked out; the reactions of Spirillum by Jennings and Crosby[1] and Rothert,[2] of Hydra by Wagner,[3] and of Planarians by Pearl,[4] and Parker and Burnett.[5] No work, however, was done by any of these authors on reactions to heat and cold in these organisms. My attention was called to this fact by Dr. H. S. Jennings, and at his suggestion and under his direction this work was taken up. I wish here to acknowledge my indebtedness to him for valuable advice and criticism.

Spirillum.

The Spirilla studied were found in cultures composed of decaying hay and aquatic plants. There were probably several species, but most of the Spirilla were thought to be S. volutans (Ehrenberg). For a brief description of this organism, its movements, chemotaxis, motor reflex, etc., see Jennings and Crosby.[6]

The thermotactic reaction of Paramecia[7] can be very clearly demonstrated, (1) by mounting the Paramecia under a large cover glass, and cooling or heating local areas with drops of cold or hot water placed on the cover glass, and (2) by first cooling or heating the slide containing the animals; then, if the slide was heated, putting drops of cold water or small pieces of ice on the cover; if it was

[1] Jennings and Crosby: This journal, 1901, vii, pp. 31–37.

[2] Rothert: Flora, 1901, lxxxviii, pp. 371–421.

[3] Wagner: Not yet published.

[4] Pearl: Quarterly journal of microscopical science, 1902, pp. 509–714.

[5] Parker and Burnett: This journal, 1900, iv, pp. 373–385.

[6] Jennings and Crosby: *Loc. cit.*, pp. 33–35.

[7] Jennings: Paper on "Reactions to heat and light," now in press.

cooled, putting drops of warm water on the cover. The slide may be conveniently heated or cooled under the microscope by placing a flat bottle containing hot or cold water on the stage and laying the slide upon it. Under the first condition the Paramecia leave the area beneath the drops on the cover; under the second they collect beneath the drops.

Experiments similar to those described above were performed, using Spirillum in the place of Paramecium, but in no case did the Spirilla collect or disperse under the drops. The only noticeable effect of a change of temperature on Spirillum was an increase in motion, both forward and rotary, when passing from regions of lower to higher temperature; and a decrease in motion when passing from higher to lower. Spirilla frequently reverse their direction of motion (motor reflex),[1] when coming in contact with solid particles or when stimulated chemically, but such reactions could not be demonstrated in the case of thermal stimuli. The organisms were subjected to both sudden and gradual changes of temperature, varying from nearly 0° to far above the ultramaximum, and indeed many were exposed to temperatures so high as to prove fatal; yet no motor reflex could be observed.

It is thus seen that while Spirilla react slightly to thermal stimuli their reactions seem in no way purposive; *i.e.*, they are not of such a nature as to keep the organisms in regions of optimum temperatures. Such non-purposive reactions were entirely unexpected, and since no similar reactions to thermal stimuli were found recorded in the literature,[2] it was thought that further investigation might prove them incorrect. In order then to carry on such further investigations I used a modification of Mendelssohn's apparatus for the study of thermotaxis.[3] By means of this apparatus temperature changes can be more accurately regulated than by the method described.

Mendelssohn's apparatus as modified in these experiments consists of two horizontal, parallel glass tubes of convenient length, with a rubber tube attached to either end. The free end of one of these rubber tubes is connected with a siphon in a jar of water situ-

[1] Jennings and Crosby: *Loc. cit.*, p. 34.

[2] Jennings personally stated later that he had obtained somewhat similar results in subjecting certain ciliate infusoria to different temperatures.

[3] Mendelssohn: Journal de physiologie et de pathologie générale, 1902, p. 409.

ated considerably higher than the glass tubes; that of the opposite rubber tube leads into a waste jar. The rate of flow of water is regulated by means of adjustable pinch cocks. Thus by heating the water in one of the outflow jars and cooling it in the other and regulating the flow, the temperature of the glass tubes can be varied at will. A slide or dish containing the organisms worked on is placed on these tubes and thus subjected to different degrees of temperature.[1]

Spirillum was mounted on a slide two inches wide and covered with a thin slide one inch wide supported by small pieces of wire to prevent crushing. A little vaseline was smeared along the edges to prevent evaporation. The slide was then laid on the glass tubes in the apparatus described above, so that one of the tubes was near either end of the slide. The temperature of one end of the slide was then very gradually lowered almost to 0°, by slowly passing water containing ice through the tube under it and finally by placing small pieces of ice on the slide over the tube; that of the other end was as gradually raised to about 50° by slowly heating the water and allowing it to flow through the glass tube under it. While these changes in temperature, which required nearly two hours, were taking place, the Spirilla remained, as nearly as could be judged, equally distributed with reference to regions of different temperatures,[2] although all in the region of highest temperature (50°) were killed before the experiment was ended. During the course of the experiment many of the organisms collected in dense groups, remained thus for a short time, dispersed, and collected elsewhere. Several such groups were formed, but these also were about equally distributed over the slide. Soon after such a group is formed a small circular area may be seen, in the centre of which there are but very few Spirilla. This area gradually becomes larger and larger until the group disappears. In the regions of comparatively high temperature the groups form more quickly and disperse more quickly than in regions of lower temperature. These aggregations are similar to those due, in the case of Paramecium,[3] to the excretion of carbon dioxide by the organisms. If in the case of Spirillum they are also due to the excretion of carbon dioxide, their more rapid formation when the temperature

[1] This apparatus is figured in the unpublished paper of JENNINGS, already cited.

[2] A BRAUS-DRÜNER stereoscopic binocular was used in studying the reactions.

[3] JENNINGS: Journal of physiology, 1897, xxi, pp. 258–322.

is higher, is what would be expected, since an increase in temperature causes an increase in activity, and consequently would cause an increase in excretion of carbon dioxide. Experiments with carbon dioxide seemed to indicate, however, that this is not the cause of the aggregations in the case of Spirillum.

A number of Paramecia which happened to be among the Spirilla collected in a region about one centimetre wide, a little nearer the cooler than the warmer end of the slide.

This experiment was performed repeatedly, and the same result obtained in each case. The results confirm in every respect those obtained by the first method. We may therefore conclude that Spirillum volutans is not thermotactic in the true sense of the word. This being true, a determination of the ultramaximum and ultraminimum temperatures will be of some interest.

The ultramaximum temperature, as above stated, was reached in the region of highest temperature in the experiment with Mendelssohn's apparatus. It was then only necessary to measure this temperature. This was done by laying on the slide bits of paraffin having different melting points, and later determining the melting point of that which melted over the region fatal to Spirillum. The ultramaximum temperature was thus found to be between 49° and 50°

In order to determine the ultraminimum temperature, a solution containing Spirillum was kept for twelve hours on ice, but without freezing. This temperature did not prove fatal. Another solution was gradually frozen and allowed to thaw very slowly while surrounded by the freezing mixture, the mixture being uncovered and set in a room at about 22°. After the ice thawed, the Spirilla were all found to be dead. It is possible that the ice surrounding the Spirilla reached a temperature slightly below 0°, but it is not probable, since as soon as the solution was frozen it was uncovered and allowed to thaw. We may thus consider the ultraminimum temperature of Spirillum volutans to be somewhat below 0°. It is of course probable that the physical changes which occur in freezing or thawing are the real cause of death.

Hydra.

In the following work Hydra vulgaris was used exclusively. The material was largely collected in the Huron River, where Hydras were found in abundance attached to pond lily leaves and other

plants growing near the head of a mill pond in water having a very slight current.

Two methods of applying thermal stimuli were used; one by increasing or decreasing the temperature of the water in which the animals were kept, the other by bringing an object of high or low temperature near the animals. In the first method, Mendelssohn's apparatus was used with modifications; for the second method an apparatus was constructed as follows: A small glass tube about fifteen centimetres long was drawn out into a capillary tube in the middle, and then bent on itself so as to form a long narrow U tube. The two arms of the U tube were then passed through holes in a cork, which served to hold the tube and also to strengthen it.

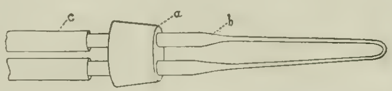


FIGURE 1. — Apparatus used in applying local thermal stimuli; *a*, cork; *b*, glass tube; *c*, rubber tube.

A small rubber tube was slipped over the end of each arm. One of these was connected with a siphon in a jar about one-half metre above the table, the other opened into a waste jar. Thus the water in the jar above the table could be siphoned through the U tube, and by regulating the temperature of the water in the jar and the rate of flow by means of a pinch cock, the temperature of the U tube could be varied as desired, and kept nearly constant even under water.

The results of applying stimulations according to the second method will be discussed first. The Hydras were put into small glass dishes containing water about seven millimetres in depth, and left till they were well expanded. The U tube was then carefully put into the water some distance from an animal, and slowly brought near its tentacles, foot, or sides. If the tube is moderately warm (50°–60°), the Hydras respond very readily to the stimulus by contracting, whatever part of the animal stimulated by the tube. As to the relation of the movement to the localization of the stimulus, the following may be noted. When the tube is brought near the foot, the animal contracts of course toward the source of stimulation. (This was found to be always true, no matter how great the stimulation.) When the tube is brought near the sides, the animal contracts in a line at right angles with the source of stimulation. In such contractions no lateral movements which would carry the animal away from the source of stimulation could be detected. Only when the tube is

brought near the tentacles from in front, do the animals contract so as to get away from the source of stimulation. If the tube is kept near the animal for some little time after it has contracted, it will frequently expand again. The direction of such expansion, however, bears no definite relation to the source of stimulation. The animal is just as likely to move toward the source of stimulation as in any other direction. Thus, on the whole, it is evident that the direction of the movements induced in Hydra by thermal stimuli has no relation to the localization of the stimulus. If the temperature of the tube is increased sufficiently, the Hydras will no longer expand, and may be killed without further movement. In no case was it possible by this method to cause the animal to release its foothold and to move away. Such movements were, however, produced by another method. (See page 171.)

If water at 0° is passed through the tube, and a Hydra treated as described above, the animal reacts essentially as if hot water had been used; but the reactions are much slower and less definite. In fact, it is often impossible to tell whether such reactions are due to a stimulus caused by a decrease in temperature, or whether they are spontaneous reactions, since a Hydra, under normal conditions, slowly contracts and expands once every two or three minutes.

The reactions of Hydras to thermal stimuli as described above agree with their reactions to chemical and mechanical stimuli as worked out by Wagner (*loc. cit.*) with one exception. Wagner found that if a Hydra is slightly stimulated mechanically for a long time, it will release its foothold and move away; but the direction of such movements, as in case of expansion after thermal stimulation, bears no relation to the source of stimulation.

In reactions due to thermal stimuli, a Hydra does not always react simultaneously throughout, *i.e.*, there may be local responses due to local stimulations. One or more of the tentacles frequently contract first, after which the rest of the animal may or may not contract. Such reactions are especially prominent when Hydra is thermally stimulated from the oral end. The body of an animal may also contract without the tentacles, but this reaction is rare. If Hydra is stimulated near the foot, the different parts of the body usually contract simultaneously.

If a well-heated tube is lightly brought in contact with the side of the body of an expanded Hydra, the animal will immediately bend at the point of contact, toward the source of stimulation, until it nearly

if not quite forms a right angle at that point. After thus bending, it soon contracts; but when it re-expands an angle is again formed at the same place as at first, though it is not so acute as the first. Thus as the animal contracts and expands the angle gradually becomes more nearly straight, until it finally disappears. Wagner (*loc. cit.*) obtained similar reactions to local chemical stimuli. He suggests that these reactions may be traumatropic, *i.e.*, due to injuries; and I am inclined to believe they are, since they cannot be produced by local mechanical stimulations or stimulations due to a decrease in temperature. If the U tube through which water at 0° is flowing is brought in contact with the body of a Hydra, it usually contracts immediately, though rather slowly, but does not bend at the point of stimulation.

The question as to the ability of Hydra to react in such a way as to protect itself against unfavorable temperatures has already been referred to, but requires further consideration. It was found by experiment with the U tube that the animals do not move away from the source of stimulation by releasing their foothold or by contracting or expanding in such a way as to avoid critical temperatures. It was thought, however, that they might do so if temperature changes were more carefully regulated. For this purpose, Mendelssohn's [1] apparatus, with modifications, was used. In place of the slide, a tin box, three centimetres deep, nine centimetres wide and twenty centimetres long was set on the two glass tubes so that one was near each end of the box. The box had a cross partition, three centimetres from one end, making a water-tight compartment. After filling the box with water to the depth of about eight millimetres, several Hydras were scattered over the bottom. The temperature of one end of the box was then gradually decreased to nearly 0° by putting small pieces of ice into the compartment above-mentioned, and that of the other end was gradually increased to 38° by slowly passing hot water through the tube under it. As the temperature increased, the rate of contraction and expansion of the Hydras also increased, and later the animals began to release their foothold and to move away from the point at which they had been attached. But the direction of such movements, as well as the direction of expansion, had no definite relation to the source of stimulation, *i.e.*, a Hydra was just as likely to move or to expand toward the source of stimulation as away from it (*cf.* Fig. 2.), so that when the ultramaximum temperature (34°) was reached the

[1] Described under Spirillum, page 166.

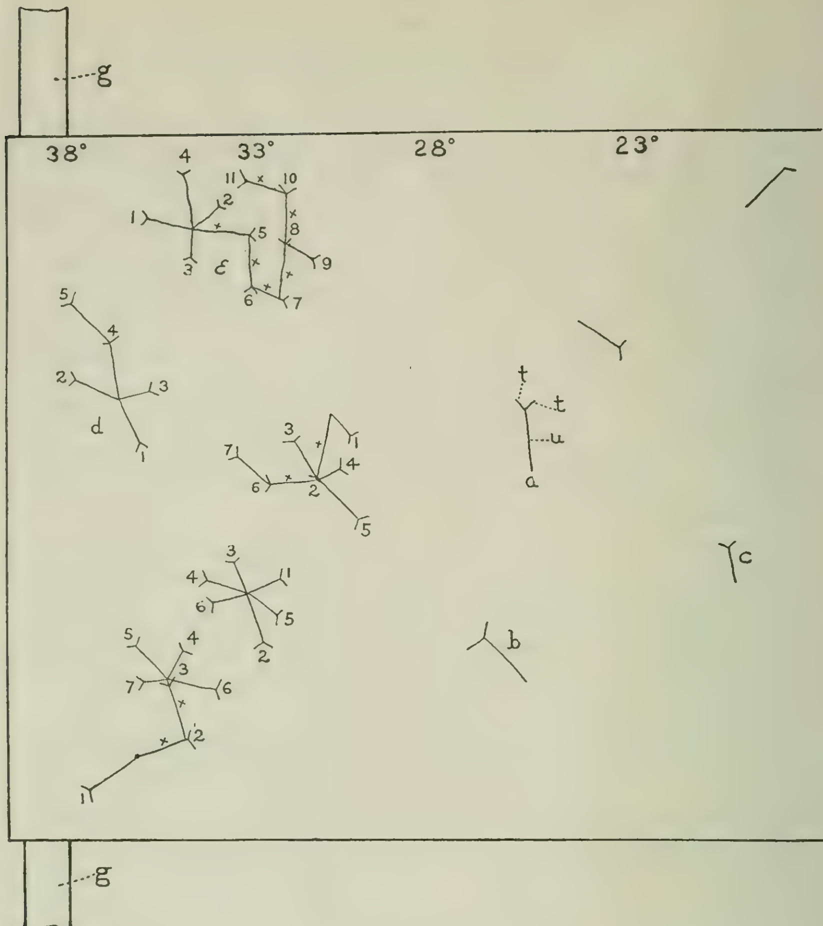


FIGURE 2. — Diagram representing the reactions of Hydra to gradual increases of temperature as seen in Mendelssohn's modified apparatus. The diagram represents one-half of the box used in connection with the apparatus. *g*, glass tube; 38°, 33°, 28°, 23°, temperatures at end of experiment. (The temperature of the right end of the box, not represented, was nearly 0°.) *a*, *b*, *c*, etc., Hydras; *t*, tentacles; *u*, body. The numbers, 1, 2, 3, etc., represent the successive positions taken by an animal as the temperature increased; the highest number in each series shows the place where the animal died. *x*, between two numbers signifies that the animal changed position by releasing its foothold. In nearly all other cases the animals changed position by first contracting and then expanding in a different direction. Not all the contractions and expansions are represented. Occasionally, however, they changed position by moving the oral end laterally. The animals represented by the diagrams without numbers changed their position by contracting and expanding several times during the experiment, as did also several others situated in regions of lower temperature in that portion of the box not represented, but none of them moved by releasing their foothold. It can be seen from this diagram that Hydras move at random, and are as likely to move toward the source of thermal stimulation as away from it; also that they move by releasing their foothold only when the temperature is increased to about 31°, and not when it is decreased.

animals died, although a movement of one or two centimetres in the right direction would have carried them out of danger. Comparing these results with those obtained by stimulating with a hot tube, it will be seen that they agree in all respects save one. By stimulating with a tube Hydra could not be forced to release its foothold, while it could by stimulating as just described. This may be due to the fact that with the U tube the foot could not be stimulated as much as the rest of the animal, since as soon as the water was heated upward currents were formed.

The Hydras in the region of lowest temperature (nearly 0°) contracted and expanded very slowly and less frequently than under normal conditions. None of these released their foothold and moved away. In performing this experiment, the results obtained are the same when the small compartment contains either ice or water at about 22°. The experiments require one to one and one half hours.

Considering the results above stated, it is difficult to see how the reactions of Hydra due to changes in temperature can be of any protective value to it, excepting in a rather accidental way. It is hardly probable that the contractions and expansions of Hydra are in any way protective against critical temperatures. The reaction which consists in releasing the foothold and moving away from the point of attachment in some random direction would, of course, occasionally result in escaping critical temperatures, even if, as shown above, the animal is as likely to move toward the source of stimulation as away from it. But Hydra reacts by releasing its foothold and moving away only in response to stimuli due to increase of temperature and not to those due to decrease, and then only if the temperature is increased to nearly 31°. Now Hydra in its natural environment is seldom if ever subjected to a temperature of 31°, consequently it is probable that few if any Hydras were ever under natural conditions thermally stimulated in such a way as to cause them to react by releasing their foothold and moving away. But practically all of them will give this reaction if properly stimulated; thus we are certain that all or almost all Hydras that give this reaction in the laboratory in response to thermal stimuli do so without previous experience. Are such reactions due to an inherent property of protoplasm; or can they be explained on the theory of "natural selection"?

It seems to me that the fact that the reaction to mechanical and

thermal stimuli are in all essential points the same, throws some light on the question.

A mechanical stimulation produces a certain definite physiological change in an animal, which causes it to respond with a definite reaction. It is evident that if the same physiological change could be produced in any other way, the animal would respond with the same reaction. In other words, similar reactions in an organism are due to similar physiological changes in the organism regardless of the kind of stimuli that have caused the changes. Then, since the reactions of Hydra to thermal and mechanical stimuli are similar, the physiological changes which cause the reactions are probably similar. If this is true, in order to explain the non-protective reactions of Hydra to thermal stimuli, it is necessary only to explain the reactions to mechanical stimuli. But the reactions to mechanical stimuli are protective, and their development in Hydra may be explained by the theory of "natural selection," that is, through frequent mechanical stimuli, which cause definite physiological changes, Hydra has, by "selection," acquired the power to respond in a certain definite way. When similar physiological changes are produced by thermal stimuli, Hydra reacts in the same way, although its reactions may be of no value. This question will recur in the account of the reactions of Planarians.

A number of experiments were performed in order to determine the degree of sensitiveness of Hydra to temperature changes. In carrying on such experiments, the animals were put into a beaker and set in a shallow dish into which hot or cold water was siphoned, and thus the temperature of the water in the beaker raised or lowered, and the number of degrees of change necessary to cause contraction noted. Conclusions derived from such experiments are subject to two sources of error. In the first place, it was many times impossible to tell whether the contractions of Hydra were due to temperature changes or whether they were spontaneous. In the second place, it was impossible to measure the temperature of the water next the walls of the beaker; and it was the change in temperature in this water, or even possibly the temperature of the walls of the beaker, which gave the stimulations. The results of all these experiments may be summed by saying that Hydras living in water at about 22° respond to a sudden increase in temperature of from 1° to 2°. If the temperature is first gradually increased to 28° or 29°, and then suddenly increased, they respond to a change of from $\frac{1}{2}$° to 1°. Reactions due to a decrease in temperature were so varied and

uncertain that it was impossible to come to any definite conclusion, but it was evident that a greater decrease than increase was necessary to produce reactions. In fact, as will be shown later, the temperature can be easily decreased so slowly as not to cause any noticeable modifications in the normal reactions.

The ultramaximum temperature for Hydra was roughly determined in the experiments with Mendelssohn's apparatus. To determine it more accurately, and also to study more carefully the effect on Hydra of gradually increasing the temperature, a beaker containing five animals was set in a medium-sized bacteria dish full of water at 22°. This water was then displaced, drop by drop, by conducting hot water into the bottom of the bacteria dish. Thus the temperature of the water in the beaker was very gradually raised; in fact, so gradually that it required fifty-six minutes to raise it from 22° to 34°. As the temperature increased, the rate of contraction and expansion also increased slightly, until a temperature of about 28° was reached, when the animals began to remain contracted longer, and did not expand so fully as under normal conditions. At 31° they began to release their foothold, and move away from the point of attachment. Such movements continued from time to time until the temperature reached 34°, when all motion ceased. It required twenty-three minutes to raise the temperature from 31° to 34°. During this time one of the Hydras moved seven times, the rest from two to five times. At the beginning two were attached to the sides of the beaker; these moved down to the bottom. Those attached to the bottom were in about the same position at the end of the experiment as they were at the beginning. In moving they stretched out partially, turned the free end down until it came in contact with the bottom so as to form an arch, raised the foot, frequently remained supported on their tentacles a minute or more, and then put the foot down near the tentacles, sometimes on the same side upon which it had been before it was raised, and sometimes on the opposite side, thus turning a somersault. None were seen to crawl on their tentacles. Two of the Hydras died while supported on their tentacles, and remained thus after death. One hour after all motion ceased, all the animals were nearly liquefied. We may thus conclude that the ultramaximum temperature for Hydra, living in a temperature of about 22°, is about 34°.

The above experiment, in connection with those performed with Mendelssohn's apparatus, seems to me to lead to another conclusion

worthy of mention. In the experiment just described, the animals were stimulated equally on all sides; while in the experiment with Mendelssohn's apparatus, they were stimulated more strongly on one side than on the other. Now the reactions under these two conditions are found to be the same. If the reactions are due to a direct effect on the motor organs, as Mendelssohn believes, in the case of infusoria,[1] we should expect different reactions under these two different conditions. And furthermore, according to the theory of a direct effect on the motor organs, it is difficult to see why Hydra, when stimulated equally on all sides, should release its foothold and move away, — a movement which requires unequal reaction in the muscle fibres on two different sides of the body, which are subjected to equal stimuli. These results seem to me to lead to the conclusion that thermal stimuli act indirectly on the motor organs, and that the direct cause of the reaction must be sought elsewhere.

If the temperature of the water in which Hydras are found is gradually decreased from 22° to 0° in about one hour, they do not appear to be stimulated. They contract and expand from time to time, as they do under normal conditions, but more slowly and less frequently as the temperature approaches 0°. At low temperatures Hydras also become much less susceptible to mechanical stimuli, and more easily loosened from their attachment by the foot than under normal conditions, so that those attached to the surface film, and some of those attached to perpendicular surfaces, fall to the bottom, frequently remaining expanded during such descent. This effect of a decrease in temperature on the attachment of the foot may be due either to a physical change in the mucus, or a physiological change in the cells of the foot. The fact that the attachment is affected in this manner by cold seems to be of some biological significance. Hydras are usually found near the surface of the water in which they live, and consequently, since a decrease in temperature does not cause them to move, they are subject to freezing, which, as will be shown later, is fatal. Now, since the attachment of the foot becomes much weakened as the temperature decreases, the slightest jar will cause them to fall to the bottom, and thus they may escape being frozen and killed.

Under normal conditions the longitudinal axes of expanded Hydras are usually nearly perpendicular to the surface to which they are attached. As the decreasing temperature approaches 8°, the animals

[1] MENDELSSOHN: *Loc. cit.*, pp. 484–485.

slowly bend near the foot so that the longitudinal axis, if not attached to horizontal surfaces, becomes vertical. This bending seems to be due to a decrease in the tonus of the muscle fibres near the foot, and consequently an inability to hold the animal perpendicular with the attached surface against gravity.

At 0° the activity of Hydra ceases almost entirely. At this temperature some may be seen fully expanded lying on the bottom as if dead; others remained contracted. But if the temperature is raised, the animals recover at once. Several, thus kept at 0° for twelve hours without being frozen, recovered as soon as the temperature was raised to normal. If the temperature of water is gradually lowered, Hydras may be frozen in a partially expanded condition. Several were thus frozen and examined in the ice. While some of them were well contracted, the bodies of others were found to be from two to five millimetres long, and their tentacles well expanded. Frozen Hydras slowly thawed in a temperature of 22° do not recover. Their ultra-minimum temperature is therefore below 0°, — death being probably due to the physical changes in freezing or thawing.

Fresh-water Planarians.

As noted in the introduction to these studies, Pearl (*loc. cit.*) and Parker and Burnett (*loc. cit.*) have carefully worked out the general behavior of Fresh-water Planarians, excepting their reaction to thermal stimuli.

A quotation from Pearl will show the extent of his work. "The general natural history of the animal was studied as completely as possible. All the normal movements were studied in detail. The reactions to mechanical stimuli; the food reactions and reactions to chemicals in general; electrotaxis; thigmotaxis; rheataxis; the righting reaction; the reaction of cut and regenerating pieces; and hydrotaxis and the reaction during desiccation were investigated. No work was done on the phototaxis or thermotaxis." A study of phototaxis was made by Parker and Burnett (:00).

Pearl in his study found that there are two principal, qualitatively different reactions to stimuli, the positive and the negative reactions.

"The negative reaction is given in response to strong unilateral stimulation of the anterior portion of the body. It consists essentially in a turning away of the head from the side stimulated.

"The positive reaction is given only in response to weak unilateral

stimulation of the anterior portion of the body. It is essentially a turning of the head toward the source of stimulation. This reaction is one of considerable precision, bringing the anterior end into such a position that it points in most cases exactly toward the source of stimulus."[1] Pearl found that the positive reaction was given in response to all weak mechanical, rheotactic, and chemical stimuli, regardless of the substance used as a stimulus, and that the negative reaction was given in response to all strong mechanical and chemical stimuli, regardless of the agent used as a stimulus with one exception.[2] The negative reaction could not be produced by rheotactic stimulations.

A brief review of some of Pearl's results has been given because in the following description frequent reference to them will be made and some of his terms will be used. His experiments on mechanical stimulations were repeated by me, and results obtained which agree with his throughout.

Material. — Planaria dorotocephala was used almost exclusively in the following experiments. The animals were collected in the Huron River at Ann Arbor immediately below a dam, in a swift current, where they were found on the under surface of rather large stones, which had settled well into the substratum.

Reactions to local thermal stimulations. — In giving local stimulations, the U tube described under Hydra (Fig. 1) was used. Quantitative results are practically impossible in using this method of stimulation. Some quantitative work, however, was done later.

If the tube, while hot water is flowing through it, is carefully brought near the margin of a Planarian, anywhere in front of the œsophagus, the Planarian turns towards the tube, — the source of stimulation, — *i. e.*, it gives the positive reaction above referred to. In thus turning the animal sometimes lifts its anterior end, and if the temperature of the tube is not too high, it usually continues to move towards the tube, and occasionally grasps it with the anterior end, as is customary in food reactions.[3] Then if the tube becomes too warm on contact, as is usually the case, the animal turns its anterior end away, — thus giving the negative reaction described above. Frequently, however, in giving the positive reaction the animal does not turn sharply enough to come in contact with the tube, merely turning toward it slightly, and passing by. If the tube is very warm, the animal usually raises its anterior end, brings it toward the source of

[1] PEARL: *Loc. cit.*, p. 700. [2] *Loc. cit.*, p. 657. [3] *Loc. cit.*, p. 32.

stimulation, and then, as it reaches a region of comparatively high temperature, suddenly throws its anterior end in the opposite direction until this frequently forms a right angle with the posterior half of the body; then gradually it swings its head toward the source of stimulation again. A Planarian will thus not infrequently swing its anterior end from side to side three or four times, as if in the act of investigating matters, and then finally move away from the source of stimulation, — giving the negative reaction. If the tube is very warm, and is brought near the anterior end of the animal rather suddenly, the negative reaction is induced, not preceded, by the positive.

In the above description use has been made of the phrase "turns towards or away from the source of stimulation." From this it must not be understood that the animal orients itself and moves parallel with the rays of radiation or convection, for this is only seldom true, and then it is apparently accidental. The question of orientation will be referred to again later (see page 184).

It is not always possible to cause a Planarian to give the positive reaction, whatever care is exercised in varying the degree of thermal stimulation. Pearl found this to be true also with reference to mechanical stimuli, and showed that whether an animal can be made to give the positive reaction or not depends upon its physiological condition, *e.g.*, animals in an excited condition or at rest will not respond with the positive reaction.[1]

It was found, however, that while this is also true with reference to thermal stimuli, the positive reaction to thermal stimuli is more definite than to mechanical stimuli, and can be more readily and more frequently produced by the former than by the latter.

Thermal stimuli on any portion of the posterior half of a Planarian cause, if weak, slight increase in gliding movement, and, if *strong*, "crawling" movements. Crawling movements can be induced only if the tube is *hot* and brought very near the animal. They were not induced by ventral stimulation. If the tube when very warm is held over the anterior end of a Planarian near the so-called oral appendages, the animal soon turns its anterior end to one side, but if the tube is now moved over the oral appendages again and kept there, the animal throws its anterior end up and twists it so that the ventral surface of the head faces the tube. When the stimulation becomes too strong, due to the fact that the head is brought too near the hot

[1] PEARL: *Loc. cit.*, pp. 592–595.

tube, the worm suddenly withdraws the head, usually by contracting the anterior portion of the body so as to get out from under the tube. It then turns aside and moves on. Sometimes, however, instead of thus contracting and backing out from under the tube, it glides forward and escapes in this way.

Ventral stimulation. — Thermal stimulation can readily be applied to Planarians gliding along the under side of the surface film with their ventral surface up,[1] or by putting them in some water on a *thin* piece of glass and heating or cooling the glass from below.

If a hot iron is held above the anterior end of a Planarian gliding along the surface film, it will throw its anterior end down (a negative reaction) and swing it from side to side. If the stimulation is continued, the animal will leave the surface film and sink to the bottom. A Planarian stimulated in the region of the head, from below, by heating the stratum upon which it is gliding, will throw its anterior end up, swing it from side to side, and if the stimulation is strong enough, finally turn abruptly and move away. If the stimulation is not very strong, the worm will pass over the heated region, after raising its head and swinging it from side to side a few (two to five) times. In such cases the animal continues in about the same direction after stimulation as it did before.

Reactions to stimulation due to a decrease in temperature. — In stimulating Planarians by decreasing the temperature, precisely the same methods were used as in stimulating by increasing the temperature; and the reactions in response to such stimuli were essentially like those in response to stimuli due to increase in temperature; *i. e.*, the anterior end was frequently raised and swung from side to side; positive reactions were given to weak stimuli and negative reactions to strong stimuli. The negative reactions to decrease in temperature were, however, not so pronounced as those to increase in temperature; while the positive reactions appeared to be more pronounced, and the head was also less frequently raised and swung from side to side in response to cold than in response to heat.

The difference between reactions to stimuli due to decrease and those due to increase in temperature is probably owing to the fact that as the temperature decreases the animals become less susceptible to stimuli. Then, too, it is possible to increase the temperature much more than it is possible to decrease it; since if the temperature of the water in which the animals live is 22°, it can be increased 78°, but decreased

[1] Pearl: *Loc. cit.*, p. 534.

only 22°. Thus one would be likely to give stronger stimuli in increasing the temperature than he would in decreasing it.

Equal, simultaneous, thermal stimuli on all surfaces. — In order to stimulate a Planarian equally on all sides at the same time, it is of course necessary to have the temperature equal on all surfaces. At first thought this appears to be a very simple matter, but in reality it is not so simple as it appears. The chief difficulty lies in the fact that Planarians move along the bottom or sides of the vessel in close contact with its walls, which, under ordinary conditions, are warmer or colder than the water in the vessel. This difficulty was at least partially overcome in the following way. A Petri dish, about two centimetres deep and twenty centimetres in diameter, without a cover, was set on three small pieces of glass, one centimetre thick, in a large bacteria dish, which was then filled with water at about 60°. Thus the Petri dish was entirely surrounded by water, and its walls soon became practically of the same temperature as the water within. After the water in the bacteria dish had cooled to a desired temperature, a Planarian was carefully transferred from water at normal temperature to the bottom of the Petri dish and the reactions studied. A section lifter was found very convenient for transferring the animals.

If the water in which the Planarians live is at 22°, and the water in the Petri dish at 32°, and an animal is transferred from one to the other, as soon as it reaches the bottom of the Petri dish it raises its head slightly (less than when ventrally or laterally stimulated) and, without forward motion, swings it from side to side five or six times violently, so that it frequently touches the tail. Then the animal starts to crawl, usually in a direction almost opposite that which it faced when first put into the dish. It usually crawls only a short distance, contracting two or three times, then glides rapidly, making frequent curves in its course. These curves, however, soon disappear, and the animal glides about in a perfectly normal way and soon comes to rest. The time required to regain normal reactions after being transferred varies with different animals from twenty-five to thirty-five seconds. If a Planarian, on the other hand, is carefully transferred from a dish containing water at 22°, to a dish containing water at the same temperature, it usually starts off at once in the direction in which it faces when it reaches the bottom. Sometimes it crawls a short distance, making two or three contractions, and then glides, but it usually glides from the start, making but few curves in its course.

If animals are transferred to the Petri dish from time to time, as the temperature decreases, it is found that their characteristic reactions, described above, become gradually less marked, until, at 25°, it is questionable in many cases if there is any difference between their reactions when transferred to the warmed Petri dish or to another dish containing water at 22°. Thus it would seem that an increase of 3° in temperature is necessary to produce a stimulus strong enough to cause a response in Planarians. This matter will be considered more in detail later (see page 188).

In analyzing the reactions of Planarians to equal simultaneous thermal stimuli on all sides, one is led to consider the cause of such reactions. Why should a Planarian swing its head from side to side when the muscles and nerve endings of both sides are subjected to the same temperature; and why make more frequent curves in its course than under normal conditions? If the reactions to thermal stimuli are due to a direct effect of the stimulating agent on the motor organs, causing either a simple contraction or expansion, we should expect neither of the above reactions. The fact, however, that these reactions are given, leads to the conclusion we deduced in the discussion on the reaction to equilateral simultaneous stimuli under Hydra (see page 175), namely, the reactions to thermal stimuli cannot be due to a direct effect on the motor organs, nor to simple single motor reflexes. Apparently the stimulus causes a change in the physiological condition of the animal, and the movements are the expression of this changed condition.

Optimum temperature. — In determining the optimum temperature for Planarians, Mendelssohn's apparatus was used, as modified in the work on Hydra (see page 171).

If the temperature at one end of the box is reduced almost to 0°, by keeping ice in the small compartment, and that at the other end raised to about 32°, and then several Planarians are scattered in a little water at the bottom of the box, the results are as follows: The animals glide about the box for a short time, and then come to rest scattered over a somewhat wide area near the middle of the box. The lowest and highest temperatures at the two opposite limits of sixteen such areas were taken. It was found that these varied considerably. The temperature limits of the area at one extreme were 10° and 16°, while those at the other extreme were 20° and 29°. The average lowest temperature of the sixteen areas was 17°, and the average highest 26°. Thus we may consider the optimum tempera-

ture of Planaria living in a temperature of about 22° to be between 17° and 26°.

Reaction by means of which Planarians reach regions of optimum temperature. — If a Planarian in its wanderings about the box happens to come near the warmer end (32°), it pauses a moment, raises its head, swings it from side to side from one to five times, usually not moving forward during this process, then moves on, after changing its course

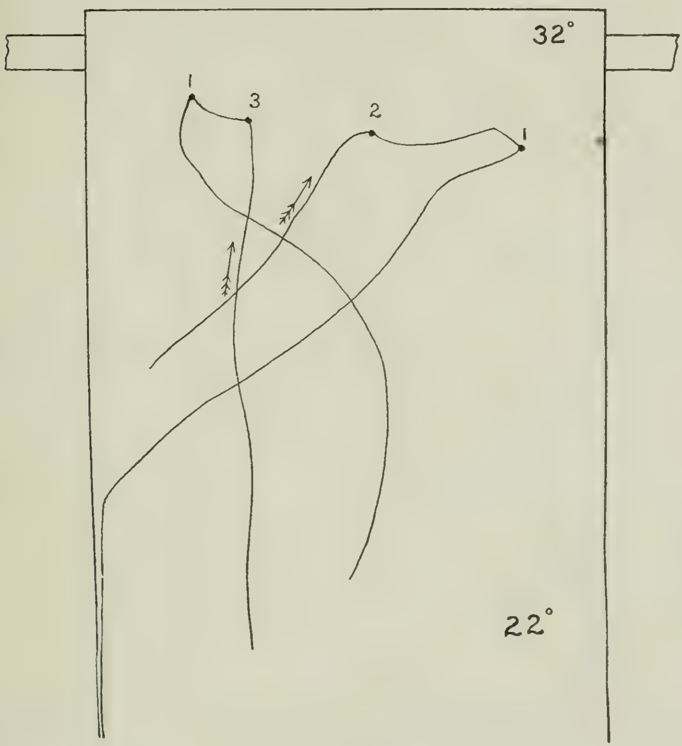
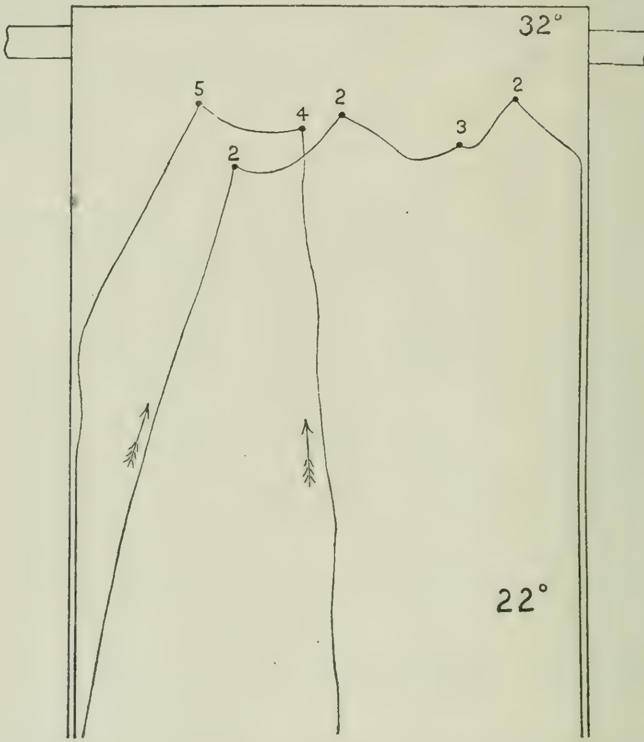


FIGURE 3.

so as to form an approximate right angle with its original course; *i.e.*, it gives the negative reaction. Its new course is then usually more or less nearly parallel with the end of the box, and perpendicular to the rays of radiation and convection. The animal does not, however, continue on a straight course, but gradually turns towards the source of stimulation; *i.e.*, it gives the positive reaction. Soon, however, it is brought to a region of comparatively high temperature, and is strongly stimulated, when it again raises its head, swings it

from side to side, gives the negative reaction, and starts off at an approximate right angle with its old course. Its anterior end may now be carried far enough away from the source of stimulation so that it will not again be stimulated sufficiently to give the positive reaction: the animal will consequently continue straight on its new



FIGURES 3 and 4. — Diagrams of the reactions and typical courses of a Planarian coming into and getting out of a region of comparatively high temperature, as seen in the box used in connection with Mendelssohn's apparatus. The temperature at one end of the box was 32°, and that at the other end 0°. The dots represent places where the animal stopped and raised its head. The small figures near the dots indicate the number of times it swung its head from side to side before taking a new course. The arrows indicate the direction of locomotion. The curves toward the warmer region show that the animal responded with the positive reaction.

course, which may form almost any angle with the rays of radiation and convection. It may, however, before leaving the region of high temperature, alternately give the negative and positive reactions several times, and in so doing move approximately parallel with the end

of the box, making several curves (see pages 183, 184, Figs. 3 and 4). An animal after leaving the warmer may go straight toward the colder end, and there give reactions similar to those described, but not so pronounced. After this it may again reach the warmer end, and give the reactions, thus continuing until it finally comes to rest in any region where the conditions are such as not to give an effective stimulation. It will be seen from this description and the accompanying figure that in reaching the optimum temperature the animals do not orient themselves and move in any definite relation to the source of stimulation or the rays of radiation and convection.

Ultramaximum temperature and reactions to gradual increase in temperature.—The method used to determine the ultramaximum temperature for Planarians was that already employed for Hydra (see page 175). If a Planarian taken from a dish of water at 23°, is put into the beaker and the temperature slowly raised, the animal gradually becomes more active, gliding about rapidly and turning frequently, but it usually soon comes to rest. The reaction given is clearly that that we have described as the positive reaction. After the temperature has increased a few degrees, the animal begins to move again, now raising its head frequently and swinging it from side to side, as if investigating. As the temperature still increases, the forward motion decreases and the lateral movements increase. The negative reaction is now very strongly marked. Finally both lateral and forward movements cease, and the animal begins to make rapid, violent, crawling contractions (crawling reaction); in this it moves forward but little. Soon after this it begins to twist the body so that it sometimes has two complete turns in it (righting reaction, Pearl). Finally it turns the anterior and posterior end under, arching the central part of the body upward. Usually the anterior end is turned under farther than the posterior, and the animal goes over forward onto its back (final reaction). Sometimes, however, it goes over sidewise. After it gets on its back, the two ends may move forward and backward a little, but the animal soon dies. It may then be concluded that the ultramaximum temperature for Planarians living at a temperature of about 24°, is approximately 42°.

We thus have, as the temperature rises and the stimulation increases, the following reactions given consecutively: positive, negative, crawling, righting, and final. (All the reactions described by Pearl (*loc. cit.*), with the exception of some of the food reactions, and

the "final" reaction in addition.[1]) The positive reaction is conspicuous between the temperatures of 23° and 26°, the negative between 26° and 38°, the crawling between 38° and 39°, the righting between 39° and 40+°, the final reaction between 40+° and 42°. These reactions were studied carefully in fourteen different animals, divided into three groups, and were found to vary surprisingly little.

The reactions of Planaria in response to a gradual increase in temperature bring up a number of interesting questions. How does it happen that to a single stimulus, not localized, we obtain successively, as it increases in intensity, such a varied series of reactions, — comprising indeed almost all those of which the animal is capable? — The general impression given is that as the thermal stimulus increases, the animal tries, in a sort of "hit or miss" way, every reaction which it has at command, in order to get rid of the stimulation. The first reaction ("positive") is that which the organism gives when subjected to any slight, non-injurious environmental change; it gives the impression of "seeking." The next reaction ("negative") is that regularly induced by more intense environmental changes, which would in the long run be injurious; it tends under usual circumstances (though not under those of the present experiment) to remove the organism from the agent affecting it. The crawling reaction is another method, perhaps still more effective, of producing the same result. The "righting" and "final" reactions, to which the organism has recourse when the stimulus becomes more intense and the previous methods of response have proved ineffective, are more difficult to interpret; under certain unusual conditions, however, they would be useful. It is remarkable that the organism gives these reactions, when others have failed, even under conditions to which they are not at all adapted.

The fact that we thus get such a varied series of responses to a single method of stimulation again indicates that the results cannot well be due to a direct action of the stimulating agent on the individual motor organs. Under the conditions of the experiment the animal is equally affected on both sides and ends of the body by the stimulating agent. Yet it responds with "positive," "negative," and other reactions, of exactly the sort that are produced under other circumstances by stimulation at one side or one end. These results reinforce our previous conclusion, that the direct result of

[1] Pearl (*loc. cit.*) describes reactions somewhat similar to the "final reactions" in his study on reactions to desiccation.

stimulation is to produce some change in the physiological state of the organism, and that the reactions given are the results of the changed physiological state. Under the influence of gradually increasing heat, the Planarian undergoes a series of changes in physiological condition, and gives the reactions corresponding to these.

The fact that practically all reactions which can be produced by mechanical, chemical, rheotactic, and thigmotactic stimuli, varied in strength and applied to different regions of the body, can also be induced by thermal stimuli varying only in intensity and not varying in the regions of application, seems to show that reactions in Planarians depend very largely on intensity, and but little upon the location and kind or quality of stimulation. All the reactions to thermal stimuli induced by gradual increase in temperature, with the exception of the negative reaction, seem non-protective, under the conditions of the experiment. Some of them are probably never induced as reactions to heat, under the natural conditions of existence. The development of such reactions in Hydra has been discussed on pages 173 and 174. While the development of the positive, negative, and crawling reactions to thermal stimuli in Planarians can be explained by applying the same line of argument, greater difficulties arise in thus attempting to explain the righting reaction and the final reaction.

Ultraminimum temperature. — If Planarians are put into a beaker of water and the temperature slowly decreased, they gradually become less active and respond less readily to mechanical stimuli, until a temperature of about 10° is reached. At this temperature they no longer respond to mechanical stimuli, and practically all motion has ceased. If the temperature is lowered still more, the animals turn the two ends under, and usually roll over onto the back ("final reaction," see page 185). They may remain an indefinite length of time at 0° without harm, providing they do not freeze; but if they are frozen, and thawed, they are killed.

Several Planarians were kept at 0° over night without freezing. The following morning most of them were found lying on the back with the two ends curled up. They were then transferred to water at 22°, and almost immediately recovered.

Three Planarians were frozen and thawed in the same beaker with several Hydras whose fate has been discussed (see page 177). The freezing or thawing killed the Planarians. We may consequently consider 0–° as their ultraminimum temperature.

Threshold of sensitiveness to thermal stimuli. — This question has already been referred to (see page 182), but it may be well to collect here all we have with reference to it.

Two methods were used in determining the degree of sensitiveness of Planarians to temperature changes. In one the animals were transferred from water in which they lived to water at various higher temperatures, until a temperature was obtained in which (when the animals were transferred to it) no difference could be seen between the reactions and the reactions given when they were transferred to water at the same temperature as that from which they were taken. In the other method, Mendelssohn's apparatus, as modified in the experiments under optimum temperature, was used and the temperature of the water over the tube increased until the animals would no longer pass over it without giving definite reactions.

The results of a large number of experiments according to both of these methods may be summarized by saying that Planarians respond to stimuli caused by a rather sudden change in temperature of from 2° to 3°. That is, if the temperature of the water to which animals are transferred is two or three degrees higher than that from which they are taken, they respond by giving definite reactions. Likewise when the water in Mendelssohn's apparatus over the tube is from 2° to 3° higher than that fifteen centimetres from it, the animals, on reaching the region over the tube, give definite reactions.

Planarians then are susceptible to stimuli produced by an increase in temperature of from 2° to 3°.

General Summary.

I. Spirillum.

Spirillum volutans is not thermotactic; that is, it does not react to temperature changes in such a way as to collect in regions of optimum temperature, or avoid regions of suboptimal or supraoptimal temperatures.

Increase in temperature causes increase in motion; decrease in temperature, decrease in motion.

The ultramaximum temperature, when the temperature is gradually increased, is between 49° and 50°.

The ultraminimum temperature is slightly below 0°.

II. HYDRA.

Hydra has two methods of reacting to thermal stimuli, one by contracting, the other by releasing its foothold and moving away from the point of attachment. The second reaction is given only in response to stimuli due to increase in temperature.

The reactions of Hydra to thermal stimuli are essentially the same as its reactions to mechanical and chemical stimuli.

The reactions to thermal stimuli cannot be explained as due to a direct effect of the stimulating agent on the motor organs.

The direction of movement in the reactions bears no definite relation to the source of increase or decrease in temperature, *i.e.*, Hydras are as likely to move toward the source of stimulation as away from it, and *vice versa*.

The reactions to thermal stimuli are not directly protective, though they may be so at times in an accidental way.

Local contractions may take place in response to local stimuli.

Hydras respond at normal temperature to an increase in temperature of from 1° to 2°. They are less sensitive to a decrease in temperature than to an increase.

The ultramaximum temperature of Hydra is about 34°, the ultraminimum is slightly below 0°.

III. PLANARIANS.

Planarians respond to weak and strong thermal stimuli, just as they do to weak and strong mechanical stimuli. That is, they turn towards the source of a unilateral stimulus applied to the anterior portion of the body, if it is weak, and away from it, if strong, and they respond by crawling if the stimulus is applied to the posterior portion of the body.

By gradually increasing thermal stimuli, applied equally on the sides and ends of the body, Planarians can be made to give all the different reactions given in response to mechanical, chemical, rheotactic, and thigmotactic stimuli of different strength and applied to different regions of the body.

The reactions of Planarians to thermal stimuli, depend, primarily, upon the intensity of the stimulus, and, secondarily, upon the physiological condition of the animal and the location of the stimulus.

– The nature of their reactions to stimuli in general bears little if any relation to the quality of the stimulus.

Planarians in their reactions do not orient themselves with reference to thermal rays of radiation or convection, *i. e.*, the path which they follow is liable to form any angle with such rays.

- Their reactions are due, apparently, to a general physiological change in the organisms, rather than to a direct effect on the motor organs or a mere simple motor reflex.

Of all the reactions induced by thermal stimuli, the negative reaction appears to be the only one that is under the conditions of the experiments directly protective or useful.

- The optimum temperature, when the temperature is slowly increased, is 42°, and the minimum below 0°.

- Planarians at ordinary temperatures react to an increase in temperature of from 2° to 3°.

THE HYDROLYSIS AND SYNTHESIS OF FATS BY PLATINUM BLACK.

By HUGH NEILSON.

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THE use of catalytic agents in accelerating chemical action has long been known. Just how and why a catalytic agent acts is not well understood. Yet the similarity of catalytic action to that of the so-called ferments or enzymes has led physiologists to teach that enzyme action is a catalytic action. This relation has been especially emphasized by Bredig in his classical work, Anorganische Fermente.

Among catalytic agents which are used for chemical purposes are finely divided metals, as platinum, gold, silver, and palladium. To this list may be added the colloidal solutions of platinum, gold, etc., prepared by Bredig. He finds in the action of these colloidal solutions, especially of platinum, a similarity to the action of certain enzymes in the splitting of hydrogen peroxide into water and atomic oxygen. This action is a catalytic action and therefore shows a relation between the action of the platinum and the enzymes. The similarity in their action is further shown by the following experiments:—

1. The oxidation of alcohol to acetic acid is brought about by the presence of Mycoderma aceti (Pasteur), or by the presence of finely divided platinum (E. Davey).

2. According to O. Sulc[1] dilute oxalic acid is decomposed by powdered palladium, platinum, etc., and also, according to Jorrissen,[2] by the presence of certain fungi.

3. Laccase accelerates the oxidation of pyrogallol. This can also be done with Bredig's colloidal platinum, as K. Ikeda has shown.

4. Schönbein[3] says that the oxidation of pyrogallol by hydrogen peroxide can be accelerated by platinum black.

[1] Sulc: Zeitschrift für physikalische Chemie, 1899, xxviii, p. 719.

[2] Jorrissen: Chemisches Centralblatt, 1898, ii, p. 1084.

[3] Schönbein: Journal für praktische Chemie, 1863, lxxxix, p. 24.

5. The inversion of cane sugar can be brought about by the action of finely divided metals, according to Rayman and Sulc.[1]

6. The decomposition of hydrogen peroxide into water and atomic oxygen can be very greatly accelerated by platinum, gold, silver, etc. (Thenard[2]), as well as by many organic ferments (Schönbein[3]).

On the one hand, oxydase splits up hydrogen peroxide into water and atomic oxygen; invertase inverts cane sugar; zymase ferments sugar. On the other hand, we find that platinum black will bring about the same reactions. The only logical conclusion is that there is a similarity in these actions; or as the action of the platinum is catalytic, so also the action of the enzymes is the same or at least similar, namely, catalytic.

In this series of experiments the similarity is carried still farther. Platinum black is used instead of the fat-splitting enzyme, lipase.

In the experiments, attempts are made to answer the following questions: —

1. Does platinum black accelerate the hydrolysis of a fat, as ethyl butyrate?

2. Is this action reversible, or will platinum black synthesize ethyl butyrate from butyric acid and alcohol?

3. Do poisons and antiseptics lessen its catalytic action?

4. Do these reactions obey the same laws of chemical kinetics as lipase?

Methods.

The experiments on the hydrolysis of ethyl butyrate were carried on in medium-sized test-tubes, while those on the synthesis of ethyl butyrate from butyric acid and alcohol, were carried on in 250 c.c. flasks. All glassware used in the experiments was carefully washed, rinsed in distilled water, and then sterilized in a hot-air sterilizer. The platinum black used was principally Merck's preparation. Before it was used in the experiment, it was washed with distilled water until the wash-water was neutral to litmus or phenolphthalin. It was then placed in a drying oven and afterwards heated to 100° C. for an hour. After this it was carefully powdered, and kept in a

[1] Rayman and Sulc: Zeitschrift für physikalische Chemie, 1896, xxi, p. 481; xxviii, p. 719.

[2] Thenard: Memoire de l'Academie des Sciences, 1818, iii, p. 385.

[3] Schönbein: Journal für praktische Chemie, 1863, lxxxix, p. 24.

desiccator. The ethyl butyrate and butyric acid were the ordinary chemically pure articles.

The experiments, except those made to determine the effect of different temperatures, were performed at a temperature of 38°–40° C. The test-tubes, with the contents, were placed in the incubator for thirty minutes, in order to get the required temperature, and then the platinum black was weighed in the desired amounts and run into the tubes through a paper funnel. These were then left in the incubator the desired period of time, and afterward placed in ice-water to stop the action as much as possible. After becoming cool, the contents of each tube was poured into a medium-sized evaporating dish. To this also was added the water in which these tubes were afterwards carefully rinsed. This was then titrated with $\frac{n}{20}$ NaOH with phenolphthalin as the indicator. Owing to the platinum black making the mixtures very dark, the phenolphthalin was used in preference to litmus as the color is brighter and the end point is thus more accurately determined.

Knowing the amount of ethyl butyrate originally present, the per cent of hydrolysis was easily calculated.

In each experiment a control was made to determine the amount of hydrolysis (if any) without the platinum black. One was also made to determine the acidity of the ethyl butyrate used. The acidity of the control, due to both these factors, *i. e.*, the small amount of hydrolysis and the acidity of the ethyl butyrate, was deducted from the acidity of the tube containing the platinum black. This difference was the amount of acid produced by the catalytic action of the platinum black.

Owing to the difficulties of measuring such small amounts of ethyl butyrate, and in order to avoid differences in the amount, a mixture was made as follows: —

200 c.c. distilled water,
10.4 c.c. ethyl butyrate,
2 c.c. 1% solution of thymol, as an antiseptic.

Five cubic centimetres of the above mixture were placed in test-tubes, together with 300 mgms. of platinum black. These tubes were then placed in the incubator, and shaken at regular intervals. This shaking was necessary, as the particles of platinum black are of comparatively large size and soon settle to the bottom of the tubes. If left in this condition the catalytic action of the platinum black was

much less than when the tubes were shaken. The shaking kept the particles in suspension longer, and therefore the typical colloidal solution of platinum, as described by Bredig, was more nearly approached.

The same amount of shaking was given the control tubes as was given those containing the platinum. An experiment was made to determine whether the shaking had any effect on the hydrolysis without platinum black. There was no increase of the acidity in the tubes shaken over that of those not shaken. Therefore we are warranted in asserting that the shaking merely keeps the particles in suspension longer, and thereby increases the surface action of the particles of the platinum.

1. **Effect of time on the hydrolysis of the ethyl butyrate.** — The temperature was 40° C., the amount of platinum black 300 mgms., and the quantity of ethyl butyrate 0.26 c.c. in 5 c.c. distilled water.

The results, with the time element taken as a variable, are shown in the following table: —

Experiment 1.			Experiment 2.		
Time in hours.	c.c. $\frac{n}{20}$ NaOH.	Per cent hydrolysis.	Time in hours.	c.c. $\frac{n}{20}$ NaOH.	Per cent hydrolysis.
2	0.9	2.4	24	4.10	10 3
8	1.55	4.0	48	11.25	28.3
24	3.54	8.9	72	14.80	37.3
32	4.39	11.0	96	19.40	49.0
48	6.55	16.5	144	27.00	68.0
72	9.22	23.3			

Other experiments show approximately the same results, but with slight differences in the amount of hydrolysis, owing perhaps to differences in the amount of shaking, variations in temperature, etc. One experiment was carried on one hundred and twenty hours, with 45.7 per cent hydrolysis. Another was carried on eight days, with 60 per cent hydrolysis. The per cent of hydrolysis calculated from the increased acidity, is to some extent due to the acetic acid formed from the alcohol produced in the hydrolysis. Therefore, the results given are not entirely due to the butyric acid produced.

The liquid from a number of experiments was distilled over the water bath. This distillate was again distilled. The second distillate contained alcohol, as was shown by the iodoform test. The liquid remaining from the second distillate had unmistakably the odor of butyric acid. It also showed a small amount of acetic acid, when the acetic ether test was used.

2. **Effect of concentration of platinum black.** — In this experiment the concentration of platinum black is a variable, while the other factors are constants.

Time, 32 hours; temperature, 40°; quantity of ethyl butyrate, 0.26 c.c.		
Platinum in milligrams.	c.c. $\frac{n}{20}$ NaOH.	Per cent hydrolysis.
25	0.35	0.8
50	0.63	1.58
100	1.17	2.94
150	2 00	5.00
200	3.00	7.50
250	3.75	9.00
300	5.35	13.40
400	6.75	17.00

Other experiments show the same or nearly the same results. Here, evidently, the catalytic action is a function of the concentration of platinum. But there is not a direct ratio, as the table clearly shows. Especially is there a greater hydrolysis than we should expect, between 250 mgms. and 300 mgms. Other experiments show that this is not constant at this period; therefore it must be due to some difference in the conditions.

3. **Effect of temperature on catalytic action of platinum.** — In this experiment, the temperature is a variable, while the time, platinum, and ethyl butyrate are constants.

In this experiment five temperatures were used: (1) 0 – 1° C., by a freezing mixture; (2) 10°, in a refrigerator; (3) 20°, by the tubes being placed in running water; (4) 40°, in an incubator; (5) 60°, in

a second incubator. The time was twenty-four hours, the quantity ethyl butyrate 0.26 c.c., and the amount of platinum black 300 mm. The results are shown by the following table: —

Temperature.	c.c. $\frac{n}{20}$ NaOH.	Per cent hydrolysis.
0	0.3	0.75
10	1.00	2.5
20	2.05	5.1
40	5.00	12.00
60	6.9	15.00

The catalytic action of the platinum in this experiment increases with the temperature. The increase from 40° to 60° is not as great as might be expected. This increase is fairly constant in other experiments and therefore cannot be a varying condition of this experiment. Possibly this fact is due to the high temperature which vaporizes the ethyl butyrate and thus removes it from the sphere of action, or possibly it may be that the action of platinum is lessened at 60°, as that of lipase is at 45°.

4. **Effect of concentration of ethyl butyrate.** — In this experiment the concentration of the ethyl butyrate is a variable, while the other factors are constants and the same as those of the other experiments. Starting with 0.5 c.c. of ethyl butyrate to 5 c.c. of water, the amount of ethyl butyrate is gradually decreased 0.0025 c.c. The results are shown in the following table: —

Concentration of ethyl butyrate.	c.c. $\frac{n}{20}$ NaOH.	Per cent hydrolysis.
5 c.c. H_2O+		
0.4 c.c. ethyl butyrate	1.83	4.6
0.2 c.c. " "	1.70	4.0
0.1 c.c. " "	1.80	4.5
0.05 c.c. " "	2.00	4.9
0.025 c.c. " "	1.75	4.2

With 0.6 c.c. ethyl butyrate, the per cent of hydrolysis was about two-thirds, as much as with 0.2 c.c. With 0.8 c.c. it was one-half as much. This is probably due to the fact that the platinum is collected in clumps by the drops of ethyl butyrate which have not gone into solution, thereby decreasing the action. This experiment shows, however, that the action of the platinum is independent of the concentration of ethyl butyrate.

5. **Action of poisons on the catalytic action of platinum black.** — Two series of experiments were made.

a. In each test-tube 5 c.c. of 1–1000 solution of each poison were placed. To this were added 300 mgms. of platinum black and 0.26 c.c. of ethyl butyrate.

b. 4 c.c. of a solution containing 200 c.c. distilled water and 10.4 c.c. of ethyl butyrate were placed in each test-tube. To this was added 1 c.c. of a 1–1000 solution of each poison and 300 mgms. of platinum black.

With the first series, the following results were obtained: —

A Series. Time, 48 hours. Temperature, 40°.			B Series. Time, 24 hours. Temperature, 42°.		
Substance.	c.c. $\frac{n}{20}$ NaOH.	Per cent hydrolysis.	Substance.	c.c. $\frac{n}{20}$ NaOH.	Per cent hydrolysis.
Water	8.40	21.0	Water	0.25	13.2
Sodium fluoride .	7.25	18.0	Chloroform . .	4.37	11.2
Formaldehyde .	7.13	18.0	Sodium fluoride .	3.75	9.5
Chloroform . .	5.18	14.5	Toluene	3.60	9.0
Toluene	4.60	11.0	Thymol	3.10	8 0
Mercuric chloride	4.38	11.0	Salicylic acid . .	3.00	7.5
Silver nitrate . .	3.88	9.7	Formaldehyde .	2.65	6.6
Salicylic acid . .	2.32	5.8	Silver nitrate . .	2.27	5.6
Phenol	1.7	4.3	Mercuric chloride	2.02	5 0
Hydrocyanic acid	0.5	1.2	Phenol	2.02	5.0
Potassium cyanide	0.0	0.0	Hydrocyanic acid	0.65	1.6
			Potassium cyanide	0.00	0.0

In each of these series the results given are the averages of several experiments. In Series A the lessened action of platinum black, in the presence of chloroform and toluene, may in part be due to the fact that the platinum black clumped together in little lumps which would lessen its surface action. Each of these substances was used in pure form.

In Series B a saturated solution of chloroform at 22° was used. 1 c.c. of pure toluene was added to the tube containing the 4 c.c. of water. The lessened action of the platinum here may be due to the fact that the toluene collecting at the surface gathered the platinum with it. The action of the poisons was not always the same as in the order given in the above tables. But the order did not vary to a large extent.

On comparing the actions of these poisons on the catalytic action of platinum black, with their effect on lipase as given by Kastle and Loewenhart, the following differences are noticed: —

Sodium fluoride is one of the most destructive agents to the action of lipase in their list. On platinum black its action is not so marked, — in fact, being little greater than the action of chloroform.

Again, hydrocyanic acid, which is very destructive to the action of platinum, is not so destructive to the action of lipase, — in fact, being only about one third as destructive as sodium fluoride. The action of other substances on platinum black approximates quite closely their action on lipase.

6. **Reversibility of the action of platinum black, as shown by the synthesis of ethyl butyrate from alcohol and butyric acid.** — The experiment was made as follows: —

A mixture of 100 c.c. of $\frac{n}{20}$ butyric acid, 40 c.c. of 20 per cent alcohol, and a little thymol, was placed in each of two 250 c.c. flasks. To one were added two grams of platinum black. This was thoroughly shaken, allowed to stand for a short time, and then the liquid decanted off, leaving all the coarser particles of platinum black in the flask. This mixture was placed in a flask the same size as the control flask and tightly sealed. A control flask was also prepared and tightly sealed. Both flasks were then placed in an incubator registering 45°, and left for eight hours. Both were shaken at intervals, and at the end of the eight hours, a distinct odor of ethyl butyrate was noticed in the flask containing the platinum, while none was noticed in the control. At the end of twenty-four hours, the odor of ethyl butyrate in the flask containing the platinum had markedly

increased, while there was still no perceptible odor in the control flask. At the end of forty-eight hours, the contents of each flask was distilled over a water bath, and 10 c.c. of distillate collected from each. This had a strong odor of ethyl butyrate in the distillate from the flask containing the platinum, but there was no odor from the distillate of the control flask. Each 10 c.c. was redistilled, and 4 c.c. of distillate collected from each. These distillates were saponified with sodium hydroxide, evaporated to dryness, and then sulphuric acid was added. The odor of butyric acid was at once noticed in the saponified distillate from the flask containing the platinum, while none could be detected in the saponified distillate from the control flask. When the second distillation was made, the distillate was poured into 200 c.c. of water, and this then distilled; as with this amount of water the ethyl butyrate comes over first, leaving the butyric acid behind.

This experiment was repeated many times with varying amounts and concentrations of butyric acid and alcohol, and always with positive results. No quantitative experiment was made to determine the amount of synthesis, owing to the large amounts necessary for such work, and the difficulty and expense of obtaining platinum black. The synthesis is evidently not so pronounced as the hydrolysis. This is also the case in Kastle and Loewenhart's work.

Conclusions.

On comparing the catalytic action of platinum black with that of lipase on ethyl butyrate, the following facts were observed: —

1. Platinum black accelerates the hydrolysis of ethyl butyrate as lipase also does. But the action of the platinum is slower.

2. The action of the platinum increases with the increased concentration of the platinum. This is also true of lipase.

3. The action increases with the temperature, reaching its maximum at 50°, which is somewhat higher than the lipase.

4. The action of platinum is independent of the concentration of the ethyl butyrate, which seems also to be true of the action of lipase.

5. Poisons with the exception of sodium fluoride and hydrocyanic acid, affect the catalytic action of platinum in a manner quite comparable to their action on lipase.

6. Platinum black synthesizes butyric acid and ethyl alcohol into ethyl butyrate, as shown by increasing odor of ethyl butyrate and saponification giving odor of butyric acid. This synthesis is also brought about by lipase.

My thanks are due Professor Loeb for his valuable suggestions in these experiments.

THE STATIC FUNCTION IN GONIONEMUS.[1]

By LOUIS MURBACH.

Introductory.

THE medusa Gonionemus, abundant during the whole summer at Woods Holl,[2] displays more definite movements than other medusæ. Certain rotation experiments made several seasons ago indicated that the animal has a sense of equilibrium that can be confused. Accepting the view commonly held, I believed this to be the function of the otocyst organs.[3] The static function in lower animals is naturally associated with the otocyst organs, which, as their name indicates, were formerly held to be auditory in function, a view now generally given up.

The otocyst organs make their first appearance in the free-swimming Cœlenterates, and are not generally present in sessile forms of animals. On account of their structure and position in medusæ, they have been called "marginal vesicles," and frequently "marginal bodies." A large number of medusæ and other free-moving invertebrates (most worms, many Crustaceæ, and all insects) do not have these organs. As to their homology, it will suffice to say that their origin differs in the groups of animals in which they are found. Even in the craspedote medusæ they originate in two ways, characterizing the two orders. Although differing widely in structure in most

[1] According to a recent note from Professor AGASSIZ the genus name of Gonionemus was derived from the words γόνυ (knee) and νέμος (grove), making the change of name recently used by some writers inapt. MURBACH: Science, 1903, xviii, p. 373.

[2] It gives me pleasure to acknowledge my indebtedness for working facilities to the Director of the Marine Biological Laboratory.

[3] In Cœlenterate literature, otocyst, statocyst, and lithocyst have been used synonymously. In this paper otocyst organ is preferred, as it is more inclusive. Many of the older authors used the name marginal bodies (Randkörper) synonymous with otocysts. In rarer cases the subumbral or marginal papillæ (Randwarzen) are also included. Otocyst organs also occur in worms, crustaceæ, molluscs, and tunicates among the invertebrates.

invertebrates, the otocyst organs agree in essentials: (1) A vesicle or sac, the otocyst — containing sensory projections; (2) foreign or secreted inorganic particles, — the otoliths. The more primitive organs are those in which the otolith is formed within the sensory basal portion of the otocyst; the next, those in which the otoliths are attached to sensory projections; and the highest, those in which delicate sensory projections alone are present.

Historical.

The true auditory function of the otocyst organs in Crustaceæ was doubted by Farre (according to Prentiss[1]) as early as 1843, but it was not until 1887 that Delage[2] was convinced from some of his experiments that they serve a static in addition to an auditory function; yet Bethe, in 1894, states that the experiments in the last half of our century have indicated that the otocysts are organs of equilibrium. The strongest evidence that they are not auditory but static, in Crustaceæ, has been given by Beer[3] and Prentiss.

In a review Lyon[4] holds that work done up to the time, except, perhaps Kreidl's, does not show that otocyst organs are necessarily static in function. In a later paper[5] he reiterates this opinion, supported by experiments.

Prentiss, especially in his excellent and exhaustive paper, combines structural and functional study, successfully repeating Kreidl's experiments with iron otolith and a magnet. He shows that up to the time of his work, three theories as to the function of the otocysts (in Crustaceæ) were held: "1. That they are purely auditory organs. 2. That they are both auditory and static in function. 3. That they are purely static in function, *i.e.*, organs of orientation." His experiments seem to leave little room for doubting the static function of the otocysts in Decapods. Thus the best evidence that the otocyst organs are static in nature has been gained from experimental work in Crustaceæ, only a few investigators in Cœlenterates having worked

[1] Prentiss: Bulletin of the Museum of Comparative Zoology, Harvard University, 1901.

[2] Delage: Archives de zoologie expérimentale et générale, 1887, v, p. 1.

[3] Beer: Archiv für die gesammte Physiologie, 1898, lxxiii, p. 1; *Ibid.*, 1899, lxxiv, p. 364.

[4] Lyon: Journal of comparative neurology, 1898, viii, p. 238.

[5] Lyon: This journal, 1899, iii, p. 86.

to the same end. Engelmann's arguments [1] in favor of the static function has no doubt influenced these.

The Hertwig [2] brothers, in their classical monograph, give a thorough discussion of the function of the otocyst organs. Up to that time two views were held as to the function of these organs in medusæ: That they are auditory, and that they are for intensifying light. Although from their morphological studies these authors decide for the auditory function, they also cite the early experiments of Romanes (1876). They correctly understood that Romanes had cut out the eye-spots in his experiments on Sarsia, and probably he did the same in Aurelia, but some confusion has arisen from the fact that he calls these "lithocysts." Haeckel had cautiously called the otocysts a general sense organ ("ein gemischtes Sinnesorgan").

Chun [3] dissected out the sensory body of Ctenophores and studied the action of the otolithic mass under the microscope. He concludes that although the organ has the structure of an invertebrate ear, yet it only regulates locomotion and does not originate movements.

Verworn [4] removed the otolithic mass in Ctenophores, either with a hot needle or by suction with a tube. In each case the animals were disoriented, but this was not the case when the underlying tissues of the otocyst were destroyed. One of his specimens regained its equilibrium after the regeneration of the otolithic mass. Although Samassa's work [5] was structural, he seems to have repeated experiments similar to Verworn's, without confirming them, for he says, "In Eucharis I was able to follow *intra vitam* the action of the otoliths, or statoliths, as Verworn calls them. . . . I can only confirm the statements of Chun." [6]

[1] ENGELMANN: Zoologischer Anzeiger, 1887, x, p. 439.

[2] HERTWIG: Das Nervensystem und die Sinnesorgane der Medusen, 1878.

[3] CHUN: Die Ctenophoren des Golfes von Neapel, 1880.

[4] VERWORN: Archiv für die gesammte Physiologie, 1891, l, p. 423. In this best case of VERWORN'S, the nervous system must have been repaired at the same time with the regeneration of the otolithic mass, and this alone might account for the result. In some experiments made on Ctenophores (Mnemeopsis) to test VERWORN'S results, it was found that injuring the sensory body (scratching or pricking with a needle) produced as much disorientation as sucking away the otolithic mass.

[5] SAMASSA: Archiv für mikroskopische Anatomie und Entwicklungsgeschichte, 1892, xl, p. 157

[6] "Ich kann CHUN'S Angaben nur bestätigen." The English equivalent does not give the precise meaning of "nur" here.

Nagel[1] believes the function of the "statolith organ" to be that of a reflex centre, and Berger[2] that the concretions in the marginal bodies have a static function.

Uexküll[3] finds "with Romanes and others" that the marginal bodies in the Scyphomedusæ are nervous centres which discharge the rhythmic contraction. He believes he has proved this in a simple fashion: After cutting away all the marginal bodies but one, the bell pulsates more slowly; if the remaining marginal body is suddenly inhibited in suitable manner, the whole bell stops instantly; upon giving it a slight movement, a new series of pulsations takes place. The author concludes that the marginal body "is an organ for the reception of mechanical stimuli." Here, as in Romanes'[4] experiments, more than one kind of sense organs and some nerve centres are involved. Although the author evidently had the otocysts in mind, the results are not as valuable as would be the case in a more definite experiment.

In a recent paper, Yerkes and Ayer[5] have ascribed a light perceptive sense to the "marginal bodies," and a static function to the tentacles. But since they describe these marginal bodies as heavily pigmented, and on the oral side of the medusa, it is probable they had in mind the subumbral papillæ.

At the present time then there are three functions ascribed to the otocyst organs in Cœlenterata: auditory, static, and reflex centres of locomotion.

Normal Activities.

It will be well to note first the activities of Gonionemus which may be concerned in maintaining its position in space.[6] When at rest on any bottom the medusa lies with the opening of the bell turned upward, or hangs suspended from some object by one or more tentacles. A single contraction of the bell throws the body away from the

[1] Nagel: Archiv für die gesammte Physiologie, 1894, lvii, p. 495.

[2] Berger: Memoirs from the biological laboratory, Johns Hopkins University, 1900, iv, p. 1.

[3] Uexküll: Mittheilungen, Zoologische Station zu Neapel, 1900, xiv, p. 620.

[4] Romanes: Jelly-fish, Star-fish, and Sea-urchins, 1885.

[5] Yerkes: This journal, 1903, ix, p. 279.

[6] The behavior of Gonionemus is so striking that one is tempted to dwell on this part. Perkins has given the most pregnant description since Agassiz's. Perkins: Proceedings of the Academy of Natural Sciences, Philadelphia, 1902, liv, p. 755.

substratum; another contraction will usually turn the slightly heavier apex of the bell, and it is driven upward. A medusa deprived of its tentacles swims with more wavering, jerky motions, but otherwise normally. By their resistance they act merely as passive regulators. The distance travelled by one contraction varies, by measurement, from 1 to 2.5 cm., until the last stroke carries one third, or more, of the aboral pole above the surface of the water. The rhythm stops, and the bell turns over, the tentacles on the under side folding close against the bell, while those on the opposite side stand out more prominently. As soon as the bell has turned, the tentacles are spread out evenly and the inverted medusa floats down. Repetition of this cycle of movements is the normal behavior of Gonionemus during its periods of activity. Some of the significant exceptions are as follows: Not infrequently the surface is not reached, yet the bell is turned; and again on reaching the surface the upward stroke may be repeated until the medusa sinks back without turning, or turns after many thrusts of the top of the bell into the air. Swimming occasionally takes place in an almost horizontal direction, or the animal may continue for some time striking the bottom without turning to rise.

Experimental.

Some experiments were made to change the centre of gravity [1] by introducing small weights into the stomach, — first, with food, and then, without. As long as the weights were not very heavy, they did not change the cycle of movements; if very heavy, the animal sank to the bottom. The movements in fluids denser than sea-water were also observed. The medusæ live several hours in a mixture of starch paste and sea-water, but suffer loss of water.

In the medusa chosen for experimentation, the otoliths are enclosed in definite sensory capsules, supported on a stalk in the interior of the otocyst.[2] The removal of the otocyst organs would be the most

[1] Repeated experiments with motionless medusæ (and freshly killed specimens) show that the centre of gravity is aboral to the margin of the bell. Some killing agents are not reliable for this purpose; *e.g.*, a one per cent solution cocaine in one quarter to two hours abstracts enough water to cause the medusæ to float aboral side up.

[2] The finer anatomy is naturally omitted. The description of the nervous system and otocysts given in "Das Nervensystem und die Sinnesorgane der Medusen," O. u. R. Hertwig, text, pp. 48–69, plates 4 and 5, fits very nearly those of Gonionemus. There is also a short account of "the Nervous system in 'Gonionema' murbachii"; Hyde: Biological bulletin, 1902, iv, p. 40.

natural beginning, but as these are near together and deep-seated, it would be impossible to remove them individually without injuring too much the adjacent nerve-ring from which they are innervated. Acids were used to dissolve the otoliths, but without success, as the medusæ always succumbed to such treatment before the concretion was attacked by the acid.

As the otocyst is a functional part of the organ, it seemed probable that destruction of the cyst would put the whole organ out of function. After some preliminary attempts, it was found to be not so very difficult to prick the vesicles with a specially ground needle.[1] As this operation had to be done under a compound microscope, considerable time was consumed, and it was rather doubtful whether it might not impair the ability of the animal to right itself. In the first medusa experimented upon only a few quick pulsations were attempted. After ten minutes it was stimulated and resulted only in fibrillar contractions. After some practice, however, the cysts were destroyed without injuring much of the adjacent tissues. Four records of the succeeding experiments are as follows: (1) Medusa swam down in the water, turned and swam upward, down two strokes, and turning over floated to the bottom. (2) Swam nearly horizontally four strokes, turned over and settled down in the characteristic way. (3) Started up spontaneously from the bottom of the aquarium, swam four strokes, and settled down again. (4) Swam four strokes slightly upward, then downward, turned up over its path three strokes, and settled to the bottom. Evidently the static function of these medusæ was not seriously impaired.

Recalling now that previous experimenters (*cf.* Bunting, Delage, Beer, Prentiss, and others) had found in other invertebrates that some organs other than the otocysts, removed along with the latter, causes disorientation, similar experiments were next begun. First, the tentacles of normal animals were removed, and it was found that orientation was not diminished.[2] The only difference noticeable was that the motions resulting from the contractions of the bell were more jerky and irregular, but by following the movements it could

[1] The vesicle collapses completely when the operation is properly performed.

[2] In a preliminary report of this medusa the author has shown that the tentacles have nothing to do with the forward propulsion of the bell (*cf.* The journal of morphology, 1895, xi, p. 493). A medusa swimming with its tentacles very much contracted has a much more irregular motion than one with tentacles flowing farther out.

easily be seen that the tentacles dragging behind serve the same purpose as the tail of the old-fashioned kite. Then the tentacles were removed from some of the animals that had their otocysts punctured. In this, as in the last case, there was no perceptible loss of orientation. The gonads were removed with no better success. The manubrium, including the walls of the stomach, has also nothing to do with the orientation. It was now recalled that in one of the author's earlier experiments the velum was severely mutilated and the animal swam, but without the ability to change its direction, and it seemed almost disoriented. Of course, if the velum is one of the principal swimming organs it would be natural for its serious injury to be attended with difficulty in swimming, but in the present case the abnormality was something more than this. The velum was now operated on in various ways to the extent of its total removal. In each case where there was serious injury to the velum, — especially mutilation, — the medusæ were unable to swim normally.[1] Animals in which the otocysts were carefully punctured and the velum removed behaved in about the same way. Similar results obtained by experimenters on other animals have been considered sufficient to prove the static function of the otocysts, but the final experiment recorded in this paper will show, it would seem, that such conclusion was not valid; even if there were not in those cases the objection of the elimination of two factors at the same time, instead of one.

If now the foregoing experiments tend to show that the otocyst organs do not, after all, take an important part in the orientation of this medusa, and mutilation of the velum does in some way make a perceptible difference, it seems probable that such regulation is brought about by "muscular sensation" of the velum. There are no special sense organs or even sense cells in the velum.

Not being satisfied with the results obtained so far, I determined to try another set of experiments.[2] These were to depend for their success on the entire removal of the otocyst organs. After numerous trials this was finally accomplished by removing portions of the margin, and after necessary repair had taken place, to remove the

[1] This would seem to support the view of YERKES and AYER that the medusa can direct its course by the velum.

[2] Besides the ordinary method of pricking the otocysts, cauterizing them with a hot needle was also tried; but this seemed to have such a detrimental effect on the surrounding tissues that it was not continued. Rearing or keeping animals in sea-water without lime salts was not attempted for twofold reason.

remaining portions. The tentacles, portions of the circular canal, and small portions of the velum were necessarily removed; then the velum was allowed to regenerate and again attach to the margin. The cutting was at first done with very fine scissors, and later with a very sharp knife, the medusa resting cap-fashion on the end of a rubber tip on the finger. The rubber served the double purpose of a non-conductor and a good cutting surface.

The difficulty of severing the velum all the way around the bell was overcome by cutting away the margin at first only perradially or interradially, leaving narrow strips adherent. From these points the velum again grew to the bell, after which the marginal portions between could be removed. In some cases the velum healed in such a way that the opening in the velum was eccentric, and this gave such striking circus movements as to suggest that a one-sided operation on some of the organs had brought this about. This defect in the velum was corrected, and the animals swam straight. It was also found that better healing followed in those cases where the hands and instruments were especially cleansed.

After an animal had healed and had become as normal as possible, judged by its swimming, it was examined with the microscope for any otocyst organs that might have been overlooked. One was found that had not been removed, and there was also at one point on the velum a small mass of independently contractile tissues, a little piece of the margin of the bell that had been cut away and had grown fast to a wound-place in the velum. Both these structures were removed, and now came the critical test. Would the spontaneity of the animal be interfered with, or its orientation impaired — perhaps be made impossible?

In about ten minutes the medusa moved almost straight upward. Twice more it was stimulated, and it moved upward near the surface of the water and sank down. One-half hour later, it turned disk upward and swam to the top twice spontaneously. The last time it turned and rested normally with the disk down.[1] Later, although the velum was very imperfectly shaped (not mutilated), the animal swam to the surface three times, twice turning of its own accord. The swimming movements were still a little one-sided, due partly to a

[1] While the pigment spots (not true "eye-spots") in the bases of the tentacles were not yet regenerated, the average time between nine spontaneous movement-intervals was taken in a northern exposure, then in the sunlight. It was twice as long in the sunlight as in the northern exposure.

slight contraction on one side of the bell, as this naturally kept the opening of the velum also at one side.[1] The defect in the velum was remedied and swimming was more normal. Tentacle buds had now appeared, though their subsequent growth was very uneven; but the otocyst organs had not made a beginning. This would seem to indicate the relative functional importance of these two sets of organs. Later, three small otocyst organs appeared on one side of the medusa. This condition was again a test of the static importance of these organs.[2] However, there was no difference between its swimming now, and at the time when all the otocyst organs were absent.

Conclusions.

If it were not now evident that the otocyst organs have nothing to do with the static function, it is at least fair to conclude that Gonionemus, perhaps all hydromedusæ of similar structure, are not materially dependent on these organs for maintaining equilibrium; furthermore, that these organs have little if anything to do with spontaneous movements.[3] Inasmuch as the experiments that have led to this conclusion have also tested other organs in this regard, it would be but a corollary to add that no other organ has this exclusive function.

It was the part of this paper only to determine the function of the otocyst organs; and their supposed function is not that of equilibration. The question naturally arises as to what is the probable means of static regulation. In the vertebrates, muscular sensation is generally accepted as playing a most important part; a number of authors give it a third or fourth place in the equilibration of many invertebrates. While, so far, no direct proof has been furnished for this view, some of the experiments make it probable that muscular sensation (largely in the velum) is the seat of static function in Gonionemus and in hydromedusæ.

[1] This one-sided contraction being so marked in the absence of the otocyst organs suggested the thought that they may, after all, have something to do with the tonicity of the muscles.

[2] It may be of interest to note that this medusa was kept in a finger bowl aquarium for five weeks. Change of water and food were given every few days, and a slight increase in size took place.

[3] The entire absence of otocyst organs in a whole group of hydromedusæ (the so-called "ocellatæ") would seem to support this contention.

THE EFFECTS OF VARIOUS SALTS ON THE TONICITY OF SKELETAL MUSCLES.

By W. D. ZOETHOUT.

IN an article[1] on the effects of potassium and calcium ions in the striated muscles, I showed that potassium chloride increases the tone of the gastrocnemius muscle of the frog, and that calcium chloride, and to a lesser extent sodium chloride, antagonize this action of potassium chloride. Subsequently I found[2] that if a potassium salt whose anion precipitates calcium is employed, the increase in tone is much greater than when the chloride is used, and that the minimum concentration of such a potassium salt necessary to cause increase of tone is much less than that of potassium chloride.

This work I continued during the summer of 1902 at the University of Chicago, and found that several other salts, such as the salts of ammonium, rubidium, and cæsium, behave like potassium salts, while the salts of strontium and magnesium are similar to calcium.

Potassium salts. — Besides potassium chloride and those salts of potassium that precipitate calcium, the effects of which are discussed in the above-referred-to articles, I tested the action of potassium iodide and potassium sulphate. Concerning the power to cause rhythmical contractions in the skeletal muscle, Loeb[3] found that sodium iodide is far more efficient than sodium chloride. This led me to surmise that a solution of potassium iodide would be more effective in producing increase in tonicity than a solution of potassium chloride of the same concentration. This I found to be true, for whether the $\frac{m}{8}$ KI be diluted with water, sodium chloride or calcium chloride solution, the increase of tone produced was always greater than that produced by the corresponding mixture of potassium chloride. While the weakest solution of $\frac{m}{8}$ KCl mixed with

[1] ZOETHOUT: This journal, 1902, vii, p. 199.
[2] ZOETHOUT: This journal, 1902, vii, p. 320.
[3] LOEB: Festschrift für Professor FICK, 1899, p. 104.

$\frac{m}{8}$ $CaCl_2$ capable of producing a slight increase in tone was found to be $4\frac{1}{2}$ c.c. KCl + $5\frac{1}{2}$ c.c. $CaCl_2$, a solution of 4 c.c. $\frac{m}{8}$ KI + 6 c.c. $\frac{m}{8}$ $CaCl_2$ called forth a considerable increase in tone.

Potassium sulphate was also found to be more active than the chloride, although this may perhaps be due to the partial precipitation of the calcium in the muscle.

Ammonium salts. — If a muscle is placed in a $\frac{m}{8}$ NH_4Cl solution, the tone of the muscle is increased, but instead of the immediate and powerful increase produced by $\frac{m}{8}$ KCl, the muscle undergoes a more gradual and limited shortening which may be preceded by a latent period varying from one-half to three minutes. Ammonium chloride is, therefore, far less powerful in its action than potassium chloride, as can also be readily seen from the following tables, which express the minimum concentration of potassium chloride and ammonium chloride necessary to cause increase in tonicity.

1 c.c. $\frac{m}{8}$ KCl + 9 c.c. H_2O	$2\frac{1}{2}$ c.c. $\frac{m}{8}$ NH_4Cl + $7\frac{1}{2}$ c.c. H_2O
2 c.c. $\frac{m}{8}$ KCl + 8 c.c. $\frac{m}{8}$ NaCl	5 c.c. $\frac{m}{8}$ NH_4Cl + 5 c.c. $\frac{m}{8}$ NaCl
$4\frac{1}{2}$ c.c. $\frac{m}{8}$ KCl + $5\frac{1}{2}$ c.c. $\frac{m}{8}$ $CaCl_2$	9 c.c. $\frac{m}{8}$ NH_4Cl + 1 c.c. $\frac{m}{8}$ $CaCl_2$

If we express these facts in terms of the molecular concentration of the potassium chloride and ammonium chloride, we obtain : —

	KCl	NH_4Cl
In H_2O	$\frac{m}{80}$	$\frac{m}{32}$
In NaCl	$\frac{m}{40}$	$\frac{m}{16}$
In $CaCl_2$	$\frac{m}{17.77}$	$\frac{m}{8.88}$

By dividing the molecular concentration of the potassium chloride by that of the ammonium chloride as given in the above table, we obtain, for dilutions with water and sodium chloride, the quotient $2\frac{1}{2}$, and for dilution with calcium chloride, the quotient 2; showing that the power of potassium chloride to cause increase in the tonicity is about twice as great as that of ammonium chloride.

From these tables it is also evident that sodium chloride and calcium chloride antagonize this action of ammonium chloride; and what is true for calcium chloride holds also for strontium chloride. I determined the minimum concentration of $\frac{m}{8}$ NH_4Cl when diluted with $\frac{m}{8}$ $SrCl_2$, and found this to be 9 c.c. NH_4Cl + 1 c.c. $SrCl_2$. Magnesium and lithium chloride also have the power to abolish the increase of tone caused by ammonium chloride.

Besides the chloride, I also tested the action of the nitrate, sulphate, succinate, and bromide. These salts are, as a rule, more effi-

cient in producing tone than the chloride, but they are also more liable to cause rhythmical contractions.

Cæsium chloride. — If a motor nerve of a gastrocnemius muscle is placed in a $\frac{m}{8}$ $CaCl_2$ solution, no contractions of the muscle take place, nor is the tone increased. In this respect this salt resembles the chlorides of potassium and ammonium; Mathews[1] found that these salts do not stimulate motor nerves. When, however, the muscle itself is placed in such a solution, the tone of the muscle begins to increase after a latent period varying from one to two minutes. This increase in tone is generally associated with small rhythmical contractions, as was first noted by Loeb.[2] However, at the time that the tone has increased considerably, the rhythmical contractions are still very small.

The minimum concentration which will induce an increase in tonicity in from four to five minutes is represented by 3 c.c. $\frac{m}{8}$ CsCl + 7 c.c. $\frac{m}{8}$ NaCl or 9 c.c. $\frac{m}{8}$ CsCl + 1 c.c. $\frac{m}{8}$ $CaCl_2$. The increase of tone brought about by this salt is also reversed by the chlorides of sodium, lithium, magnesium, strontium, and calcium.

Rubidium chlorate. — A $\frac{m}{8}$ solution of rubidium chlorate has no effect on the motor nerve. If, however, the muscle is placed in such a solution, there is an immediate and powerful increase in tonicity. The power of rubidium chlorate to increase the tonicity is of about the same magnitude as that of potassium chloride, as can be seen from the following table, which gives the minimum concentration.

$$1 \text{ c.c. } \tfrac{m}{8}\ RbClO_3 + 9 \text{ c.c. } \tfrac{m}{8}\ NaCl, \text{ or,}$$
$$4\tfrac{1}{2} \text{ c.c. } \tfrac{m}{8}\ RbClO_3 + 5\tfrac{1}{2} \text{ c.c. } \tfrac{m}{8}\ CaCl_2.$$

As pointed out by Loeb,[3] rubidium salts give rise to rhythmical contractions, but it should be noticed that the great increase in tone which takes place immediately after the muscle is immersed in rubidium chlorate is far more apparent and takes place much sooner than the rhythmical twitches.

A few experiments were made with rubidium chloride which proved that this salt has the same action as the chlorate. The tone induced by rubidium chloride or chlorate is reversed by calcium chloride and strontium chloride, and to some extent by sodium chloride and lithium chloride.

[1] Mathews, A. P.: Science, 1902, xv, p. 492.
[2] Loeb: Festschrift für Professor Fick, 1899, p. 104.
[3] Loeb: *Ibid.*

Sodium salts. — A $\frac{m}{8}$ solution of sodium chloride has no effect on the tone of the gastrocnemius muscle.[1] In some of the experiments the muscle was allowed to remain from three to five hours in the solution without the slightest increase of tone being perceptible. I have already shown that a sodium chloride solution abolishes the increase in tonicity produced by potassium, cæsium, ammonium, and rubidium salts.

In a $\frac{m}{8}$ NaI solution, the muscle may or may not increase its tone to a very slight extent, with a latent period varying from ten to

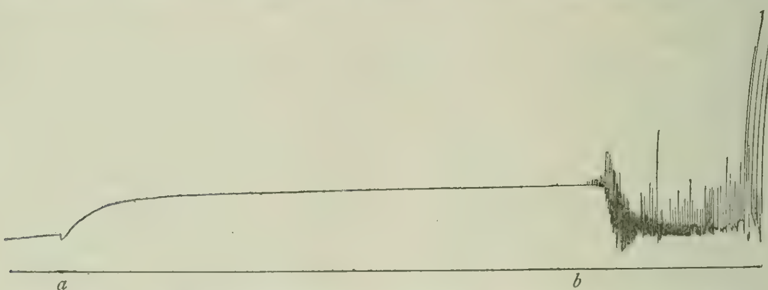

FIGURE 1. — At *a* the muscle is placed in $\frac{m}{8}$ NaI. Time between *a* and *b* is seven minutes.

thirty seconds. Sometimes this increase in tone is immediately accompanied by rhythmical twitchings, but in most cases the twitches do not set in until the muscle has been immersed in the sodium iodide from six to twenty minutes. In such cases the previous slight increase of tone may disappear with the onset of the twitches, as Fig. 1 illustrates. In this figure the muscle was placed in the sodium iodide solution at *a*, and the tone is slightly increased; the rhythmical contractions began seven minutes later (at *b*) and almost immediately the increase of tone which had existed up till this time was abolished. In some cases the sodium iodide did not produce any increase in tone, although rhythmical contractions were present.

The slight increase in tone which may be developed by sodium iodide is reversible by the chlorides of sodium, lithium, calcium, and strontium. Even the tone induced by a $\frac{m}{4}$ solution of sodium iodide is almost instantly reversed by calcium chloride. If a muscle is first treated with calcium or strontium chloride, the subsequent application of sodium iodide never increases the tone nor produces rhythmical contractions.

[1] *Cf.* ZENNECK: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 21.

As I stated in a former paper,[1] when a muscle is first treated with a sodium salt whose anion precipitates calcium, the subsequent application of potassium chloride causes a much greater increase of tone than when the muscle is directly treated with potassium chloride. I now found that this is to a certain extent also true for sodium iodide. A muscle which had been subjected to the action of $\frac{m}{8}$ NaI for ten minutes, and which showed little or no increase of tone, was at the expiration of that time placed in a bath of $\frac{1}{2}$ c.c. $\frac{m}{8}$ KCl + $9\frac{1}{2}$ c.c. $\frac{m}{8}$ NaCl, and a great increase of tonicity resulted. The strength of the potassium chloride solution used in this instance is far below the minimal strength required by the normal muscle. When potassium chloride is used after the application of sodium iodide, the twitches which may have been caused by the sodium iodide are abolished by the potassium chloride at the same time that the tone is increased.[2]

Sodium bromide acts upon the muscles in much the same manner as the iodide, but its action is less pronounced. A muscle in $\frac{m}{8}$ NaBr generally increases its tone a very little almost as soon as it is immersed. This increase in tone may or may not be accompanied by fibrillar twitchings. After a period varying from five to fifteen minutes, rhythmical contractions appear.

The tone produced by sodium bromide is reversed by calcium chloride or strontium chloride, and to a lesser extent by sodium

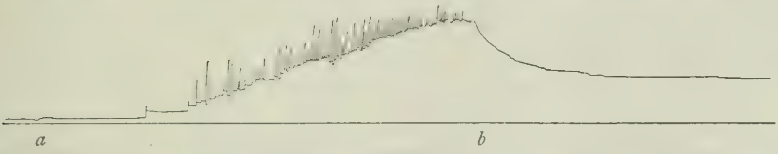

FIGURE 2. — At *a* the muscle is placed in $\frac{m}{8}$ NaBr. At *b* this is replaced by $\frac{m}{8}$ CaCl.

chloride. In those cases where the rhythmical contractions are accompanied by tone, it was observed that generally the rhythmical contractions ceased very speedily on the application of calcium chloride, while the reversal of tone was a far slower process. This is well illustrated in Fig. 2. At *a* the muscle was placed in a $\frac{m}{8}$ NaBr solution. After seventeen minutes (at *b*) this was replaced by calcium chloride. It will be noticed that while the twitches cease instantaneously, the relaxation of tone proceeds but slowly. If a

[1] ZOETHOUT: This journal, 1902, vii, p. 320.

[2] *Cf.* LOEB: Archiv für die gesammte Physiologie, 1902, lxxxxi, p. 255.

muscle is placed in sodium bromide for three or four minutes, its irritability towards potassium chloride is increased.

Of all the sodium salts tested, the sulphate causes the greatest increase in tone. If a muscle is placed in a $\frac{m}{8}$ Na_2SO_4 solution, the tone is immediately increased. In some cases this increase is considerable, in others it is moderate. The tone is generally accompanied by twitchings, and is reversible by sodium chloride, magnesium chloride, lithium chloride, and better by calcium chloride and strontium chloride.

Sodium nitrate has but little power to increase the tone of skeletal muscles. In about half the experiments, sodium nitrate induced a very slight increase, in the others none at all. If tone is developed, it is abolished by sodium chloride and calcium chloride. The irritability of the muscles towards potassium salts is increased by sodium nitrate.

Sodium asparginate causes no increase in tone. In one experiment the muscle was left in a $\frac{n}{10}$ solution for $5\frac{1}{2}$ hours without any shortening of the muscle being observed.

Neutral sodium butyrate and formate, having osmotic pressure equivalent to a 0.7 per cent NaCl solution, have little or no effect on the tonicity of skeletal muscles.

Lithium salts. — The action of $\frac{m}{8}$ LiCl is very similar to that of NaCl; it does not increase the tone of skeletal muscles. Even the increase in tonicity produced by the action of $\frac{m}{2}$ LiCl for five minutes is exceedingly small. In two experiments with $\frac{m}{4}$ LiCl there was no shortening of the muscle in ten minutes.

From this it would appear that lithium chloride, like sodium chloride, ought to counteract the action of potassium salts. This we found to be so; in fact, the power of lithium chloride to abolish the effects of potassium chloride is slightly greater than that of sodium chloride. If one gastrocnemius muscle is treated with 2 c.c. $\frac{m}{8}$ KCl + 8 c.c. $\frac{m}{8}$ NaCl, while the other is placed in 2 c.c. $\frac{m}{8}$ KCl + 8 c.c. $\frac{m}{8}$ LiCl, the contraction caused by the former solution is greater and has a shorter latent period than that caused by the latter solution. This is also proved by placing one muscle in $\frac{m}{8}$ LiCl for fifteen minutes, while the other is placed for the same length of time in $\frac{m}{8}$ NaCl; when the two solutions are exchanged for 2 c.c. KCl + 8 c.c. NaCl, the contraction of the muscle previously treated with sodium chloride is greater than that of the muscle treated with lithium chloride.

Lithium chloride also reverses the increase in tone produced by

sodium sulphate, cæsium chloride, rubidium chlorate, ammonium chloride, and other salts.

The bromide, iodide, and nitrate of lithium also act very similarly to the corresponding sodium salts; their action, however, is less powerful, and is preceded by a longer latent period. After a latent period varying from twenty to fifty minutes, the fibrillar twitches set in. They gradually increase in intensity, and are sometimes accompanied by a very slight increase in tonicity. This is removed by the use of calcium and strontium chloride. Although these salts have practically no tendency to increase the tone, yet, like the sodium iodide and nitrate, they increase the contraction brought about by a minimum concentration of potassium chloride.

This difference in the behavior of the salts of sodium and lithium appears nowhere to better advantage than in the sulphates. While sodium sulphate causes an immediate and distinct increase in the tone, lithium sulphate has no effect till after a latent period of from twenty to thirty minutes, when there is a gradual and small increase in tonicity. In a few cases, it is true, a small increase took place after a latent period of one or two minutes, but these are exceptions. The twitches induced by lithium sulphate are also small compared with those brought about by sodium sulphate. The chlorides of calcium, strontium, magnesium, and sodium reverse the tone caused by lithium sulphate.

Magnesium chloride. — This salt is very similar to calcium chloride in its action on muscle tissue. A muscle placed in $\frac{m}{8}$ $MgCl_2$ undergoes no change in tone for a long time. After a period varying from one-half to one hour, the tone is gradually increased, but in such a solution the muscle soon loses its irritability.

Like calcium chloride, the magnesium chloride counteracts the effect of potassium chloride, and its power in this respect is about the same as that of calcium chloride, for the minimum concentration of potassium chloride, when diluted with magnesium chloride, is the same as when mixed with calcium chloride. Magnesium chloride also abolishes the tone induced by salts of ammonium, cæsium, and rubidium, and it also stops the twitches produced by sodium sulphate, sodium fluoride, lithium sulphate, etc.

Barium chloride. — Barium chloride in some respects resembles calcium and strontium chloride, but in others it differs very greatly from these salts. Like calcium and strontium chloride, the barium chloride antagonizes the power of potassium chloride to increase the

tonicity. A mixture of 7 c.c. $\frac{m}{8}$ $BaCl_2$ + 3 c.c. $\frac{m}{8}$ KCl in two cases produced no increase of tone, and in a third case a slight increase after a latent period of twenty-six minutes. From this we would expect that barium chloride itself had little or no power to cause increase in tone. A slight tone may be developed in a $\frac{m}{8}$ $BaCl_2$ solution in ten or fifteen minutes, but, compared with potassium chloride, or even ammonium chloride, this action of barium chloride is very limited. In one experiment a $\frac{m}{4}$ $BaCl_2$ solution produced no shortening of the muscle in ten minutes, and in the same length of time a $\frac{4m}{10}$ solution caused but a small increase in tonicity. It is needless to say that in such solutions the muscle loses its irritability very speedily.

As was first shown by Ringer,[1] and afterwards by Loeb,[2] barium chloride causes great rhythmical contractions. In some cases the muscle in $\frac{m}{8}$ $BaCl_2$ may exhibit powerful rhythmical contractions without the tone of the muscle being increased, the lever descending to the base line after each contraction. In the other cases the rhythmical contractions may be accompanied by some increase in tone. Not only is the rhythmical contraction continued, as Loeb found, for a greater length of time in a $\frac{m}{16}$ than in a more concentrated solution, but the twitches also appear sooner in the dilute solution. Ringer also found that potassium chloride abolishes the rhythmical contractions induced by barium chloride. This I fully corroborated. A gastrocnemius muscle was placed in a solution composed of 7 c.c. $\frac{m}{8}$ $BaCl_2$ + 3 c.c. $\frac{m}{8}$ KCl, and for forty-one minutes no rhythmical contractions appeared, although after thirty-one minutes the tone was slightly increased. The control muscle was placed in a bath composed of 7 c.c. $\frac{m}{8}$ $BaCl_2$ + 3 c.c. $\frac{m}{8}$ NaCl, and in twelve minutes this muscle was thrown into powerful rhythmical twitches which were maintained to the end of the experiment.

Strontium chloride. — A $\frac{m}{8}$ solution of strontium chloride does not increase the tone of the muscle, except after the muscle has been in the solution for one or two hours. As the muscle has lost all or nearly all of its irritability when it has remained in the strontium chloride for that length of time, the shortening of the muscle is no doubt a phenomenon of rigor mortis. Even a $\frac{m}{2}$ $SrCl_2$ solution produced a comparatively small increase of tone in five minutes. In a few cases this tone was reversed by the application of sodium chloride,

[1] RINGER: Journal of physiology, 1886, vii, p. 291.

[2] LOEB: Decennial Publications of the University of Chicago, 1902, x, p. 4.

provided the strontium chloride had not acted for too long a time.

Strontium chloride acts similarly to calcium chloride, in that it prevents or removes the tone caused by the salts of potassium, cæsium, ammonium, and rubidium. The concentration of the strontium chloride needed to overcome the action of these salts is about the same as that of calcium chloride.

Calcium salts. — As already described under the effects of potassium, ammonium, cæsium, and other salts, calcium chloride has the power to prevent or abolish the increase of tone induced by these salts. A $\frac{m}{8}$ solution of calcium chloride has very little or no tendency to cause increase of tone. In fact, a muscle placed in such a solution, as a rule, shows no change until after one or two hours, and as the calcium chloride rapidly destroys the irritability of the muscle, it is more than likely that such changes are due to mortiferous processes. A $\frac{m}{2}$ solution causes a gradual shortening of the muscle, which begins almost as soon as the muscle is placed in the solution.

Calcium sulphate has a far greater tendency to increase tone. In comparing $\frac{n}{32}$ $CaSO_4$ with $\frac{n}{32}$ NaCl, the increase in tone produced by the calcium sulphate in fourteen minutes was very great, while that produced by the sodium chloride for the same length of time was nil.

General Considerations.

From the above experiments, it is evident that the various salts may be classified with reference to their ability to increase the tonicity of the skeletal muscle, as follows:

$$\text{Class I}\begin{cases}KCl\\RbCl\\CsCl\\NH_4Cl\end{cases}\qquad\text{Class II}\begin{cases}NaCl\\LiCl\\BaCl_2\end{cases}\qquad\text{Class III}\begin{cases}CaCl_2\\SrCl_2\\MgCl_2\end{cases}$$

In this table we have only included the chlorides. In Class I are placed the salts which in $\frac{m}{8}$ concentration produce an almost immediate increase in the tone of the muscle. The power of potassium chloride and rubidium chloride to increase tone is about twice that of ammonium and cæsium chloride.

In $\frac{m}{8}$ concentration, the salts in Class II do not increase the tone. In fact, as we have seen, they counteract the effects of salts in Class

I. The power of LiCl to antagonize the action of salts in Class I is slightly greater than that of sodium chloride. The salts in Class III have the same effect as those of Class II, but their antagonistic action to the salts of Class I is far greater than that of sodium chloride and lithium chloride. Another reason why I have not grouped the chlorides of calcium, strontium, and magnesium with Class II is because of the fact found by Loeb,[1] that while salts of sodium and lithium produce rhythmical contractions, the salts mentioned in Class III stop such activity. As to rhythmical contractions, it may be repeated that Loeb found that rubidium chloride and cæsium chloride, grouped by us in Class I, also have this power. The action of barium is somewhat peculiar. As already stated, its power to counteract the effects of salts of Class I is about the same as that of calcium, strontium, and magnesium; but, unlike these salts, it does not abolish the rhythmical contractions induced by the sodium and lithium salts; in fact, it augments these contractions.

We have, therefore: (1) salts which increase the tone, but do not cause rhythmical contractions (potassium chloride); (2) Salts which cause both tone and rhythmical contractions (cæsium chloride, rubidium chloride); (3) Salts which do not cause tone, but which do cause rhythmical contractions (sodium, lithium, and barium chloride); and (4) Salts which neither increase tone nor cause rhythmical contractions (the chlorides of calcium, strontium, and magnesium).

These facts would almost seem to indicate two different contractile substances[2] in the skeletal muscle, as held by Bottazzi[3] for the cardiac muscle. The stimulation of one of these, it would seem, produces a tonic contracture, that of the other produces rhythmical twitches. The first-named contractile substance, it may be assumed, is readily affected by salts of Class I in such a manner that a tonic shortening of the muscle takes place, and it is affected by salts of Class II, and especially by those of Class III, in the opposite manner. The other contractile substance is stimulated by salts of Class II, and also by the chlorides of rubidium and cæsium, and this action is prevented or abolished by salts of Class III.

The supposition of the existence of two such contractile substances is still further strengthened by facts very similar to those

[1] Loeb: Festschrift für Professor Fick, 1899, p. 104.

[2] Loeb: Archiv für die gesammte Physiologie, 1902, lxxxxi, p. 258.

[3] Bottazzi: Journal of physiology, 1897, xxi, p. 1.

that led Bottazzi to hold this view in regard to the cardiac muscle. I refer to those phenomena in which the muscles may at one time exhibit tonicity without rhythmical activity, to be followed by a period of ever-increasing rhythmical action and simultaneous decrease in tonicity (see Fig. 1).

Whether the effect of these different chlorides which possess such varied and even antagonistic actions must be assigned to the anions or the cations, I am hardly prepared to state. In regard to this question, however, I may once more refer to the peculiar action of barium chloride in conjunction with potassium chloride. As already stated, while this salt, like the chloride of calcium and strontium, inhibits or abolishes the increase in tonicity produced by potassium chloride, yet its power to induce rhythmical contractions is even greater than that of other members of Class II. And the rhythmical contractions induced by barium chloride are, in turn, inhibited by potassium chloride. As both these salts are chlorides, as the one causes tonicity, while the other causes rhythmical contractions, and as both salts antagonize each other, it seems to me most likely that we must ascribe these stimulating and inhibiting effects to the cations, barium and potassium.[1]

SUMMARY.

1. The salts of potassium, cæsium, ammonium, and rubidium increase the tonicity of the skeletal muscles. The iodide, bromide, and sulphate have a greater effect than the chloride.

2. The chlorides of sodium and lithium, and especially those of calcium, strontium, and magnesium abolish this increase in tonicity.

3. Certain salts of sodium, such as the iodide, bromide, and sulphate, may increase the tone; but such action is generally exceedingly slight compared with their power to cause rhythmical activity. The action of the lithium salts is still less than that of the sodium salts.

4. Barium chloride antagonizes the action of potassium chloride in preventing tonicity, and potassium chloride antagonizes the action of barium chloride in preventing rhythmical action.

[1] *Cf.* LOEB: Archiv für die gesammte Physiologie, 1902, lxxxxi, p. 248, and the Decennial Publications of the University of Chicago, 1902, x, p. 10.

SOME EFFECTS OF THE RÖNTGEN RAYS ON THE DEVELOPMENT OF EMBRYOS.

By P. K. GILMAN and F. H. BAETJER.

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THE great interest aroused by the discovery of the marked alterations produced in the human skin by exposure to Röntgen rays has given rise to a number of experiments on animals. This work, however, has been confined in the main to a study of the lesions produced in the skin of adult mammals [1] and has not been much extended to the lower animals and to embryos. F. Schaudinn,[2] found that various protozoa were differently affected by exposure to the Röntgen rays. Those having a protoplasm very rich in water seemed to be the ones most greatly affected. Recently Bohn [3] has shown that radium causes deformities and arrest of development in frog embryos.

In the following paper a brief account is given of the gross effects noted in certain embryos exposed to the X-rays. A study of the microscopical alterations produced in the tissues is at present under way.

Eggs of Amblystoma were subjected to the influence of Röntgen rays for fifteen minutes each day for varying periods. The eggs, just covered with water, were exposed in a shallow glass dish, and were disturbed as little as possible in transferring them to and from their tank. Both exposed and control eggs were subjected to the same conditions of temperature, daylight, and water-supply.

The first effect of the rays noted, on comparing exposed with normal eggs, was an accelerated development for a short period.

This continued up to the tenth day in the case of some of the

[1] A good summary of the literature on this subject may be found in the recent work of PUSEY and CALDWELL: "The Practical Application of the Röntgen Rays in Therapeutics and Diagnosis," Philadelphia, 1903.

[2] SCHAUDINN, F.: Archiv für die gesammte Physiologie, 1899, lxxvii, p. 29.

[3] BOHN: Comptes rendus de l'academie des sciences, 1903, cxxxvi.

embryos, though after the third or fourth day the development was seen to be along abnormal lines. About the tenth or eleventh day the normal embryos caught up in size with the exposed ones, and from that time on continued to enlarge, while the abnormal ones assumed grotesque shapes, and either grew no larger or became smaller. The experiments were continued up to the twenty-second day.

Animals exposed daily to the rays for four or five times, and then allowed to develop undisturbed, showed a marked tendency to recover and develop normally; but in less than half of these was complete restitution of form effected. Animals exposed daily for twenty-two or three days died soon after the last exposure.

Up to approximately the twelfth day the exposed animals were noticeably larger than the control. After this period practically no increase in size was noted. Although the shapes assumed by the exposed embryos were not exactly the same, the following deformities were noted in practically all of a large series, ranging from specimens of the tenth to those of the twentieth day: (1) no external gills developed on any of the embryos; (2) the body surface appeared roughened and wrinkled in places, especially about the head portions, which were slender, elongated, and more pointed than the normal heads, and showed poorly developed eyes and distorted mouth parts; (3) the bodies of the exposed animals all showed hemispherical bulgings at the base of the neck on the ventral surface, over which the body-wall appeared tense and of a lighter color; (4) the membraneous portion of the tail was but slightly developed.

Hens' eggs were employed in another series of experiments. The same care in regard to control material was observed. The eggs were exposed each day for ten minutes, and the following points observed.

The exposed eggs showed accelerated development during the first thirty-six hours, after which the eggs of this series were retarded by the rays. Specimens were preserved each day for histological study.

The chicks showed the following features on exposure for four days, the abnormalities becoming more marked on longer exposure to the rays: (1) there were deformities of the occipital region, accompanied by hemorrhagic areas, which often extended along the dorsal line; (2) the development of the eyes was retarded in many of the specimens; (3) the membranes were generally quite firmly

adherent to the embryo, so that it was difficult or impossible to remove them; (4) the limbs assumed grotesque positions, sticking out at unnatural angles to the body. In older specimens, where feathers had begun to appear, these were abnormally distributed in patches. The bodies of the exposed chicks were distorted, and many of them found hanging in abnormal positions within the shells.

In the experiments above described, the embryos were exposed to very powerful rays. The apparatus used was a twenty centimetre coil with an interrupter of the electrolytic type and a ten-inch coil with a mechanical interrupter. Two styles of tubes were used, — a Queen, self-regulating with heavy anode, and a Heinze with a medium heavy anode. The vacuum of the tubes was so arranged that a light of medium soft quality was obtained; *i.e.*, when the hand was held before a fluroscope the flesh was very pale, and the bones were a dark gray, standing out in a sharp contrast to the flesh.[1]

With such a light a hen's egg held eighteen inches from the tube casts a gray shadow upon the fluroscope. By means of the self-regulating tubes, the desired degree of penetration was readily maintained.

Sufficient current was sent through to keep the anode at a bright red heat. The eggs were placed six inches from the anode, and were arranged each day so that each egg received the same amount of light.

Dr. Bardeen, at whose suggestion the work was undertaken, informs us that in some preliminary exposures of hens' eggs to the rays, he found only those eggs affected which were exposed to rays of considerable intensity. In a series of experiments carried on at the Zoological Station at Naples, he was unable to obtain positive effects on the eggs of certain sea-urchins and teleosts, when they were exposed to rays of moderate intensity. It is, therefore, a matter of considerable importance to determine the intensity of the rays, and the length and frequency of exposure necessary to bring about marked alterations in animal tissues.

[1] Our thanks are due to Dr. Henry M. Hurd for the use of the X-Ray apparatus belonging to the Johns Hopkins Hospital.

THE EFFECTS OF IONS ON THE DECOMPOSITION OF HYDROGEN PEROXIDE BY PLATINUM BLACK.

By C. HUGH NEILSON and ORVILLE H. BROWN.

[*From the Hull Physiological Laboratories of the University of Chicago.*]

DR. A. P. MATHEWS[1] has shown that certain salts have a stimulating effect on nerves, while others in the same concentration have a depressing effect. He ascribes the stimulating action to the negative ion or anion, and the depressing action to the positive ion or cation. When the anion of the salt solution used is more powerful than the cation, stimulation takes place; when the cation is more powerful, depression results. Bredig[2] has found that the splitting of hydrogen dioxide into water and oxygen by his platinum solution is accelerated by the hydroxyl ion, and retarded by hydrocyanic acid, cyanogen iodide, mercuric chloride, certain acids, antiseptics, and some salts. Dr. A. C. Crofton,[3] working in this laboratory, has shown that in the catalysis of hydrogen dioxide the action of a nucleo-proteid from the liver was greatly accelerated by the addition of certain substances, as salicylates and dilute alkalies. Other substances, as nitrates and cyanides, greatly diminished the action. Kastle and Loevenhart[4] very recently published papers on the decomposition of hydrogen peroxide by platinum black and other substances. They also show that certain substances inhibit, while others accelerate the catalysis. The above facts led us to try a series of experiments on the action of salt solutions on the rate of splitting hydrogen dioxide into water and oxygen by platinum black, with the view of determining the effect of the anion and cation respectively. We used the sodium salts of many inorganic and organic acids to test the effect of the anion, and the chlorides of the alkalies, alkaline earths, and some heavy metals to test the effect of the cation on the rate of the decomposition of hydrogen

[1] MATHEWS: Science, 1903, xvii, pp. 729–733.

[2] BREDIG: Anorganische Fermente, Leipzig, 1901.

[3] CROFTAN: Medical record, 1903, lxiv, pp. 6–11.

[4] KASTLE and LOEVENHART: American chemical journal, 1903, xxix, pp. 397–437 and 563–588.

dioxide by platinum black. The platinum black has the advantage that it acts purely as a catalyzer, and thus all possibility of having the reaction hidden by union of the salts with the proteid constituents in the enzyme is eliminated. The analogy between the action of the enzymes and platinum black in splitting hydrogen peroxide has been shown by many investigators, especially by Bredig; hence the value of any results obtained in the catalysis of hydrogen peroxide by platinum black in the presence of certain salts.

Methods. — To measure the amount of oxygen given off during any interval of time, wide-mouthed bottles of 200 c.c. capacity were used.

TABLE I.

Salt used.	$\frac{n}{8}$ solution.		$\frac{n}{64}$ solution.		$\frac{n}{512}$ solution.		Control.	
	Cubic centimetres of oxygen set free in							
	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.
Potassium chloride	4	15	5	15	10	24	15	32
Ammonium "	2	5	5	15	17	32		
Lithium "	2	7	8	20	10	25		
Calcium "	6	12	6	17	11	25		
Strontium "	6	11	9	21	15	30		
Barium "	2	9	9	20	14	28		
Magnesium "	3	6	5	18	11	24		
Cobalt "	..	..	8	21	13	27		
Aluminium "	..	..	5	15	9	20		

The rubber stopper was fitted with a delivery tube leading to the receiving vessel, which was an eudiometer tube of 50 c.c. capacity, graduated in $\frac{1}{10}$ c.c. In every experiment four bottles were used, three containing 25 c.c. of the solution to be tested, and a fourth containing 25 c.c. distilled water; the fourth served for control. In each bottle was placed 5 c.c. hydrogen dioxide, which was kept in ice-water. In order to get the same amount of platinum black in each bottle, the platinum black was put into distilled water, which was constantly stirred so that the platinum was suspended evenly. While the stirring was continued, 5 c.c. of this mixture was placed in each bottle. The corks were rapidly placed in the bottles, and the amount of oxygen given off was read at intervals of one and two

minutes. Three tubes were used, so that any variations in the amount of substance taken could be seen in the different quantities

TABLE II.

Salt used.	$\frac{n}{8}$ solution.		$\frac{n}{64}$ solution.		$\frac{n}{512}$ solution.		Control.	
	Cubic centimetres of oxygen set free in							
	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.
Sodium chloride	4	10	8	22	12	29	15	32
" bromide	1	3	2	5	5	10		
" nitrate	10	20	11	23	15	29		
" hyposulphite	9	16	8	18	8	19		
" butyrate	15	33	25	46	18	37		
" chlorate	19	35	11	29	..	..		
" succinate	16	36	26	45	17	31		
" acetate	16	30	16	38	14	31		
" sulphite	17	31	15	35	10	29		
" lactate	19	38	20	38	14	33		
" benzoate	18	36	18	41	20	35		
" acid carbonate	12	28	16	43	16	29		
" tungstate	20	40	25	44	18	33		
" fluoride	20	35	17	40	16	33		
" sulphate	21	37	15	26	10	25		
" tartrate	20	35	20	37	20	38		
" salicylate	20	38	20	35	15	37		
" acid phosphate	25	46	15	36	17	33		
" citrate	25	46	27	49	14	30		
" valerianate	29	48	27	48	20	37		
" oxalate	30	55	25	45	24	40		
" formate	37	50	35	50	13	33		

of gas given off. The error in the reading due to the short interval of time in fitting the stoppers was very small.

Effect of the cation. — The chlorides were selected to get the effect of the positive ions. Three different concentrations of each solution

were used, $\frac{n}{8}$, $\frac{n}{64}$, and $\frac{n}{512}$, respectively. A typical experiment is recorded in Table I. In the column for controls, the general average is recorded as the controls were uniform.

From Table I, it is seen that the salt of the alkali, alkaline earths, and the heavy metals used, uniformly cause a depression in the rate of catalysis. As the strength of the solution diminishes, the rate of catalysis is increased. Nearly normal action is allowed in the $\frac{n}{512}$ solution.

Effect of the anion. — The sodium salts of many of the inorganic and organic acids were selected to test the effect of the anion. Three concentrations of solutions, $\frac{n}{8}$, $\frac{n}{64}$, and $\frac{n}{512}$, were employed. All of the solutions used were practically neutral to litmus. The results are shown in Table II.

It will be noticed that the salts of the inorganic monobasic acids, with the exception of the fluoride, have a depressing effect on the platinum black catalysis. The hyposulphite also has a slight hindering action. Sodium bromide is seen to depress the catalysis more than any of the other sodium salts. It is at least an interesting coincidence that bromides are recognized by pharmacologists as general and typical depressants. The acid carbonate depresses slightly; the sodium sulphate has an accelerating effect in strong concentration; the acid phosphate and the salts of the organic acids accelerate the catalysis. The formate, valerianate, oxalate, and citrate are the most active. Sodium citrate is one of the most powerful accelerators, and this may possibly be related to its power of stimulating diuresis. There was no constant variation in the rate of catalysis due to the different concentrations of solutions used. But as a rule the $\frac{n}{64}$ solutions were the most active. Papers will soon be published by us containing the results of similar investigations on the effect of ions on the action of a watery extract of the pancreas in splitting hydrogen peroxide and ethyl butyrate.

We are deeply indebted to the criticism of Dr. Stewart and other members of the staff of the Hull Physiological Laboratory.

Conclusions.

In the decomposition of hydrogen peroxide by platinum black, the cation, in general, has an inhibiting or depressing effect, and the anion has an accelerating effect.

CONCERNING THE FORMATION OF SUGAR FROM LEUCIN.

By J. T. HALSEY.

[*From the Pharmacological Laboratory in McGill University.*]

ONE of the questions which still remain unsolved in the physiology of carbohydrate metabolism concerns the production of sugar from proteid. From the work of various authors, especially of Lusk,[1] we know that in certain conditions this production may amount to as much as sixty per cent of the proteid involved. In explanation of this phenomenon, two hypotheses have been formulated. One hypothesis presupposes a large nitrogen-free portion of the proteid molecule which is split off from the rest, and changed more or less directly into dextrose; the other would explain the formation of sugar from proteid by synthesis.[2]

The view that sugar is derived from proteid through synthetic processes has recently received new support in the results of experiments by Stiles and Lusk,[3] and by Knopf.[4] The former have found that dogs with phlorhizin glycosuria are able to convert a large portion (about 40 per cent) of the end-products of proteid hydrolysis into dextrose. The experiments of the latter showed that under like conditions dogs can manufacture dextrose from asparagin.

Leucin, which forms so large a portion of the products resulting from proteid hydrolysis, is naturally a constituent to which attention would first be directed in this connection. Kossel[5] and Müller[6] were the first to point to this substance as a possible mother-substance for

[1] LUSK: This journal, 1898, i, p. 395.

[2] For discussion of these hypotheses, see CREMER and LANGSTEIN: Ergebnisse der Physiologie, erster Jahrgang, Theil I, 1903, pp. 99 ff. and pp. 872 ff.

[3] STILES and LUSK: This journal, 1903, ix, p. 380.

[4] KNOPF: Archiv für experimentelle Pathologie und Pharmakologie, 1903, xlix, 123.

[5] KOSSEL: Deutsche medicinische Wochenschrift, 1898, p. 58.

[6] MÜLLER and SEEMAN: *Ibid.*, 1899, p. 209.

sugar. Cohn[1] believed that his experiments with fasting rabbits indicated that leucin was a "glykogen-bilder."

Halsey,[2] working with phlorhizin dogs, was unable to show that this was the case.

A further investigation of this point seemed desirable, and in the last two years experiments have been made in the Pharmacological Laboratory of McGill University, in the endeavor to obtain additional

EXPERIMENT 1.

Mongrel bitch, weight about 15 kg. Has fasted 50 hours. Received 1.5 gms. phlorhizin every six hours during the experiment.

Period.	Dextrose in gm.	Nitrogen in gm.	D : N	Diet.
1. 36 hrs.	72.00	15.40	4.67	Fasting.
2. 24 hrs.	49.09	14.00	3.50	"
3. "	42.72	13.38	3.20[1]	"
4. "	57.67	15.87	3.63	100 gms. egg albumin.
5. "	53.93	15.75	3.42	95 gms. " "
6. "	34.86	9.53	3.67	Fasting.
7. "	51.72	15.26	3 39	104 gms. nutrose.
8. "	46.48	14.21	3.25	100 gms. "
9. 12 hrs.	14.64[2]	4.17	3.50	Fasting.
10. "	14.64[2]	4.17		
11. "	15.00[2]	5.35	2.80	27 gms. leucin.
12. "	15.00[2]	5.35		
13. "	16.58	5.19	3.19	Fasting.
14. "	17.41	4.59	3.75	"

[1] Urine fermented in bladder.
[2] Analyzed together, and reduced to 12 hours basis.

light on this subject. The method of experimentation followed was that elaborated by Lusk, and made familiar through his various papers

[1] COHN: Zeitschrift für physiologische Chemie, 1899, xxviii, p. 211.

[2] HALSEY: Sitzungsberichte der Gesellschaft zur Beförderung der gesammten Naturwissenschaften zu Marburg, 1899, p. 102.

on phlorhizin diabetes. Precautions were observed to avoid errors through incorrect feeding, loss of urine, and other mishaps which would tend to obscure results. The urine was drawn off regularly by catheter; analyses were invariably made in duplicate. The sugar was determined both by polarization and by reduction. Lehmann's[1] method was employed in place of the more usual Allihn method, it having proved in the author's experience more rapid and convenient, and not less exact, than Allihn's. The leucin was prepared from digestion mixtures, according to usual methods, and was most carefully purified by repeated recrystallization. That used in experiment 1 was prepared from the copper salt.

Five experiments were successfully completed; in these leucin was fed six times. Twice (Exps. 2 and 3) the results pointed to a possible production of dextrose from the leucin. The conclusions were,

EXPERIMENT 2.

Mongrel bitch, weight about 15 kg. Has fasted 36 hours. Phlorhizin, 1.75 gms. every 6 hours.

Period.	Dextrose.	Nitrogen.	D : N	Diet.
1. 36 hrs.	56.21	8.96	6.24	Fasting.
2. 12 hrs.[1]	15.67	3.73	4.20	"
3. 12 hrs.	15.67	3.73		"
4. 12 hrs.[1]	24.08	6.28	3.96	Leucin, 25 gms.
5. 12 hrs.	24.08	6.28		
6. 12 hrs.	19.5	4.93	3.90	Fasting.
7. 12 hrs.[1]	10.51	2.72	3.86	"
8. 12 hrs.	10.51	2.72	....	"

[1] Analyzed together, and reduced to 12 hour basis.

however, rendered uncertain, in one case by a sudden alteration in the metabolism (Exp. 1), in another (Exp. 3) by the development of kidney lesions which made it difficult to draw positive conclusions. In four experiments, the results indicate that no sugar was manufactured from the leucin fed.

The ingestion of 27 gms. of leucin (Exp. 1) was followed by so

[1] LEHMANN: Archiv für Hygiene, 1897, xxx, p. 267.

slight an increase in excretion of dextrose that the results cannot be held to show any production of sugar from leucin. The nitrogen excretion, increased more than 3.0 gms. in Periods 11, 12, and 13, indicates that the leucin was absorbed.

In Exp. 2 the ingestion of the leucin was followed by a sudden increase in both dextrose and nitrogen excretion. If we subtract from the nitrogen excreted in Periods 4, 5, and 6, 17.49 gms., 2.7 gms., as leucin nitrogen, we have as remainder derived from body proteid about 14.8 gms. nitrogen, which, multiplied by 4.00,[1] the D : N ratio, gives us 59.2 gms. dextrose as the amount of dextrose corresponding to 14.8 gms. nitrogen. 67.6 gms. were excreted, giving us an excess of dextrose of about 8 gms., which could have been produced from the leucin fed. The extraordinary increase in the metabolic processes (Periods 4, 5, and 6), however, casts doubt upon such an interpretation as being the only tenable one.

EXPERIMENT 3.

Fox terrier bitch which has fasted 48 hours. Phlorhizin, 0.75 gm. every eight hours, subcutaneously.

Day.	Dextrose.	Nitrogen.	P_2O_5.	D : N	Diet.
	grams	grams	grams		
1	18.0	4.96	1.23	3.63	Fasting.
2	28.8	6.74	1.13	4.27	"
3	29.25	7.525	1.13	3.89	300 gms. meat.
4	25.5	7.22	1.13	3.53	" "
5	**31.0**	**8.46**	1.10	3.65	300 gms. meat, 11.5 gms. leucin.
6	**26.5**	**8.40**	1.21	3.15	300 gms. meat.
7[1]	21.0	7.18	1.12	2.92	" "

[1] Dog distinctly ill; vomits about half his food; urine contains albumin.

During the 5th day about 5.5 gms. more dextrose was excreted than on the preceding day, but at the same time the nitrogen excretion rose 1.20 gms. on this and the following day; whereas only 1.2 gms. leucin nitrogen were fed. If we consider days 5 and 6 together, we find 16.86 gms. nitrogen and 57.58 gms. dextrose excreted. Subtracting from the 16.86 gms. nitrogen, 1.2 gms. contained in the leucin, we

[1] Average of 4.20 and 3.86.

have left 15.66 gms. nitrogen, as nitrogen derived from body proteid. This 15.66 gms. nitrogen, multiplied by the D : N ratio (3.6), gives us 56.37 gms. dextrose, as the amount of dextrose which we would expect to find in the urine derived from the proteid decomposed, as indicated by the nitrogen excreted. The difference, about 1.2 gms., is so small as to be within the limit of analytical error.

The fact that on the 7th day the dog gave unmistakable evidence of impaired kidney function prevents our judging whether or not this interpretation be justifiable.

EXPERIMENT 4.

Irish terrier bitch, fasted 60 hours after receiving 2.5 gms. of phlorhizin by mouth. During the experiment 0.5 gm. phlorhizin in alcoholic solution was administered, subcutaneously, each day at 6.30 A. M., and at 2.30 and 9.30 P. M.

Day.	Period.	Dextrose.	Nitrogen.	D : N	Diet.
		grams	grams		
1	1. 24 hrs.	50.35	7.11	7.07	Fasting.
2	1. 12 hrs.	19.00	4.845	3.91	"
	2. 12 hrs.	18.46[1]	....[1]	3.58	"
3	1. 3 hrs.	8.04	2.24	3.58	Leucin, 9.5 gms.
	2. 3 hrs.	6.90	2.16	3.19	Fasting.
	3. 3 hrs.	5.40	1.548	3.48	"
	4. 3 hrs.	7.54	2.504	3.01	"
	5. 12 hrs.	4 09%[2]	1.1%[2]	3.72	"

[1] During the night the dog had a loose movement of the bowels, but 115 c.c. urine was obtained from the bladder at 6.30 A. M. In this second portion, dextrose and nitrogen were determined quantitatively, and in the contaminated portion the dextrose was determined polariscopically.

[2] Dextrose and nitrogen determined in urine obtained from bladder, 150 c.c. An earlier portion contaminated by fæces.

During the two fasting periods of the second day, the dog excreted 19.0 gms. and 18.46 gms. of dextrose. In the first twelve hours of that day, 4.845 gms. of nitrogen were excreted. In the twelve hours following the feeding of leucin, 27.88 gms. dextrose and 8.452 gms. nitrogen were excreted. Taking the figures of the preceding day for comparison, these amounts represent a plus of dextrose of about 9 gms.; of nitrogen, about 3.6 gms. Of this surplus nitrogen not

more than 1.0 gm. can be looked upon as leucin nitrogen, leaving 2.6 gms. nitrogen to be considered as body nitrogen. Taking 3.6 as the D : N ratio, this nitrogen represents 9.36 gms. of dextrose, more than enough to cover the surplus dextrose of the twelve hours. This calculation would indicate that in this experiment no dextrose was formed from the leucin fed.

EXPERIMENT 5.

Spaniel bitch, weight about 8.5 kg. Fasted 140 hours. Phlorhizin given subcutaneously in alcoholic solutions.

Day.	Period.	Dextrose.	Nitrogen.	D : N	Phlorhizin.	Diet.
		grams	grams			
1	....				0.5 gm. at 7 A.M. and 6 P.M.	Fasting.
2	....	2.24%[1]	0.652%[1]	3.44	0.5 gm. at 9 A.M. and 2.30 and 9.30 P.M.	"
3	6–9 A.M., 3 hrs.	5.10	1.50	3.40	0.2 gm. at 6 A.M.	"
	9–12 M., 3 hrs.	5.20	1.49	3.50	0.2 gm. at 9 A.M.	Leucin, 5 gms. at 8.30, 5.7 gms. at 10.30.
	12–3 P.M., 3 hrs.	4.68	1.47	3.18	0.2 gm. at 12 M.	Fasting.
	3–6 P.M., 3 hrs.	4.38	1.48	2.96	0.2 gm. at 3 P.M.	"
	6 P.M.–6 A.M., 12 hrs.	16.45	5.35	3.07	0.2 gm. at 6 P.M. 0.7 gm. at 9 P.M.	"
4	6–10 A.M., 4 hrs.	5.92	1.73	3.42	0.4 gm. at 6 A.M.	"
	10–2 A.M., 4 hrs.	6.7	2.40	2.79	0.4 gm. at 10 A.M.	Leucin, 6 gms. at 10.15. 7 gms. at 12, 12 gms. at 11.30.
	2–6 P.M., 4 hrs.	5.02	1.93	2.60	0.4 gm. at 2 P.M.	Fasting.
	6–10 P.M., 4 hrs.	5.52	1.60	3.45	0.4 gm. at 6 P.M.	"
	10 P.M.–6 A.M., 8 hrs.	10.16	3.02	3.36	1.0 gm. at 10 P.M.	"

[1] Urine obtained from bladder used for analysis.

In this experiment the ingestion of 10.7 gms. leucin on the third day, and of 25 gms. leucin on the fourth day, has produced few or no noticeable effects on the excretion of sugar. The increase in sugar in Period 2 of the fourth day is too small to be of significance when compared with the amount of leucin fed, 25 gms. In this experiment no diarrhœa occurred during or after the experiment. On the third day after 10.7 gms. leucin were fed, 35.81 gms. dextrose and 11.29 gms. nitrogen were excreted; on the fourth day after 25 gms. leucin, 33.32 gms. dextrose and 11.28 gms. nitrogen were excreted. The absence

of any evidence in our figures for the complete absorption of the leucin is regrettable. The desirability of continuing the experiment for another twenty-four hours was not recognized until too late. Nevertheless the fact that the dextrose excretion did not rise after the leucin feedings stands out prominently in the table.

Two other experiments in which leucin was fed, failed to show any evidence of a production of sugar from the leucin, but the interpretation of the results is made difficult by the occurrence in one case of kidney disease, in the other of contamination of the urine by fæces.

Although our experiments do not allow us to express too positive an opinion, we believe that they indicate that leucin, when fed in a pure form to phlorhizin dogs, is not changed into sugar.

There still remains the possibility that the leucin complex, as it exists in proteid, may be concerned in the formation of sugar, or that when leucin is fed with the other end-products of digestion, as in the experiments of Lusk and Stiles, it, together with some other substance or substances, plays a rôle in the synthesis of sugar.

I take pleasure, at the close of this article, in thanking Messrs. W. E. Ainley and J. C. Fyshe, of the class of 1904, and Messrs. F. J. Tees, F. A. C. Scrimger, and E. T. F. Richards, of the class of 1905, of the McGill Medical College, for their assistance in carrying out these experiments.

LOCALIZATION OF THE RESPIRATORY CENTRE IN THE SKATE.

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CONTENTS.

Introduction.

VULPIAN[1] and Steiner[2] studied the respiratory centre in fishes. They concluded that it is situated in the posterior limit of the medulla, and that a lesion in this region produces an immediate arrest of all respiratory action. When placed in water after such an operation, the fishes remain immovable, respiratory movements are not resumed, and soon all vital functions cease.

In my paper on the "Nervous Influences on the Respiratory Mechanism in Limulus,"[3] I have demonstrated that each ganglion is the relay station for the sensory and motor nerves of the corresponding segment. The ganglia of the œsophageal ring differ from those of the abdominal cord, especially in that the motor nerves of the former innervate muscles that cause definite movements of the mouth parts of the corresponding segment, while those of the cord innervate movements of the gills of the proximal segment. Any number of

[1] Vulpian, A.: Physiologie générale et comparée du système nerveux, Paris, 1886.

[2] Steiner, J.: Die Functionen des Centralnervensystems und ihre Phylogenese, 2 Abt., die Fische, Braunschweig, 1888.

[3] Hyde, I. H.: Journal of morphology, 1894, ix, p. 347.

these ganglionic stations could be isolated, not only from each other, but from the rest of the central nervous system, without losing their identity in function. In short, the hypothesis of a superior centre controlling the co-ordination of the parts that combine in the respiratory act in animals as low as Limulus was conclusively disproved. The view that in such animals a part of the brain, corresponding to the medulla of higher animals, governs the respiratory movements by superior control, and thus regulates, through subsidiary guards, the mechanism of respiration, must also be considered erroneous.

Investigations on respiration have been pursued in my laboratory by several of my students whose work is about to be published. H. Z. Ewing [1] has experimented on the localization of the respiratory centre, and O. H. Brown and L. W. Roller,[2] on the effects of gases and pressures upon respiration in the Acrididæ, especially upon grasshoppers. They have proved that the relative position of the respiratory centre in the central nervous system of the Acrididæ is practically the same as in Limulus. They have also found that the respiratory activity of one or more segments persisted, if the sensory and motor connection between the ganglia and the muscles controlling the inspiratory and expiratory movements of the segments was not injured. It was possible, therefore, to cut one of the Acrididæ into several breathing segments, each having a definite rhythm and force of action. Moreover, the movements of these segments are increased, decreased, or stopped when exposed to the influences of different gases and pressures, and they also respond to stimuli in the same manner as do the segments in the intact animal, when it is placed under the same conditions and subjected to the same stimulations.

Thus the evidence secured from a study of these two types, Limulus and the Acrididæ, lead to the conclusion that their respiratory movements are segmental processes, each ganglion controlling the peripheral organs of its own segment.

In the higher vertebrates not only have whole systems of nerves been lost and new ones developed, but the simple primitive relations have been changed by distortion, due to shifting and overlapping. Proximal ganglia have thus been separated, and through changes in position and environment, their connections are difficult to trace, even with the aid of morphological and neurological study. In the head

[1] Ewing, H. Z.: Kansas University Science Bulletin, 1904, ii, No. 11.

[2] Brown, O. H., and Roller, L. W.: Kansas University Science Bulletin, 1904, iii, No. 1.

for instance, certain segments have developed more than others; predominating thus in size, they overlie more distant as well as nearer related ones. Under these conditions it becomes difficult to determine the exact ganglia which originally had close relations, and which together controlled the respiratory mechanism.

The phrenic nuclei, regarded as the segmental ganglia of the diaphragm, belong to the ganglia that control a certain part of the respiratory movements. They perform a function in some respects similar to that of the ganglia which control, for instance, the movements of the gill arches or spiracles in the skate. Any one of these ganglia, as was proved in my work on the skate, can be isolated from the other centres governing the respiratory movements, without sacrificing its power of sustaining rhythmical contractions of the muscles governing the activity of the segmentally related arches or spiracles. But judging from Porter's [1] researches on mammals, the phrenic nuclei, unlike the more primitive segmental ganglia of invertebrates, or the ganglia that control the movements either of the spiracles or gill arches in the skate, represent in higher vertebrates subsidiary centres that do not possess the power of discharging rhythmical contractions of the diaphragm, excepting through impulses passing to them from a superior respiratory centre in the medulla. Their functions were possibly altered during the process of phylogenetic change, and they may, therefore, have sacrificed some of the properties characteristic of primitive segmental ganglia.

A survey of the results and conclusions reached by different physiologists who have carried on investigations relating to the respiratory centre in mammals, shows that the controlling power of the respiratory movements is believed to be collections of ganglia and nerve fibres, connected chiefly with the root fibres of the fifth, seventh, ninth, and tenth nerves.

Belief in the existence in vertebrates of a superior centre in the medulla, controlling, through subsidiary centres, the rhythmical, co-ordinating respiratory activity, seems, from the facts presented by many experiments, justifiable, though there are physiologists who still believe, with Brown-Séquard[2] and Langendorff,[3] that co-ordinated, rhythmical respiratory movements executed by thoracic and abdomi-

[1] PORTER, W. T.: Journal of physiology, 1895, xvii, p. 455; PORTER and MUHLBERG: This journal, 1900, iv, p. 334.

[2] BROWN-SÉQUARD: Journal de la physiologie, 1860, ii, p. 153.

[3] LANGENDORFF, O.: Archiv für physiologie, 1880, p. 518, 1888, p. 283.

nal muscles are possible after severing the cord from the medulla, and who therefore do not consider the respiratory centre to be wholly confined to the medulla.

If we assume that the segmental control of the respiratory movements prevails among invertebrate forms, and that the co-ordinated rhythmical contractions of the inspiratory and expiratory muscles in mammals are initiated in the brain, the question at issue is, might we not find in the lowest vertebrate types a transitional stage of respiratory control, bridging the gap from the simple segmental to the complex central apparatus existing in the brain? The respiratory centre in the skate represents primitive segments that have during developmental changes come to occupy definite areas in the medulla. The relation of the ganglia to their segmental structures, although altered is not in these and related forms so obscured by intrusion of neighboring tissue as to make it difficult to trace their connections. It is hoped, therefore, that a study of the respiratory centres in the skate may contribute toward a better understanding of the innervation of respiration in higher vertebrates.

Description of the Medulla and the Respiratory Movements of the Skate.

The medulla and its nerves. — The brain of the skate (Fig. 2), lying in its cartilaginous cranium, is easily exposed without loss of blood. It consists, as is well known, of large olfactory lobes, extending antero-laterally from the pros-encephal, a comparatively small diencephal, a mesencephal with its optic lobes, and a large cerebellum that overhangs the fourth ventricle of the medulla.

By carefully removing the cerebellum, the medulla (Fig. 3), with its dorsal lobes and nerves, is well exposed. At its antero-lateral border is a convoluted ridge, the lobus lineæ lateralis, continuous with the cerebellum and mid-brain. Posterior to, and partly covered by the lobus lineæ lateralis, is a slight protuberance, the tuberculum acusticum. It borders the posterior and lateral side of the fourth ventricle. The floor of the fourth ventricle is marked by a distinct median suture, at each side of which is a spindle-shaped ridge known as the fasciculus longitudinalis posterior. Laterad to this, is the lobus vagi, which bounds the lateral and posterior limit of the fourth ventricle. It lies close to the tuberculum acusticum, and extends anteriorly to about the middle of the lobus lineæ lateralis. In some

specimens, the lobus vagi of both sides meet in the median line, forming thus a U-shaped ridge.

The large fifth and seventh nerves are seen at the anterior lateral edge of the medulla, near the median plane of the lineæ lateralis. Ventral to them is the eighth nerve, consisting of three branches. Lying close to the lateral border of the tuberculum acusticum, may be seen the ninth root, extending posteriorly to join the four root fibres of the vagus. The last-named four roots emerge on a level with the posterior end of the fourth ventricle. Each of these roots has its special ganglion at its exit at the side of the medulla, as is well shown in Herrick's[1] illustration of the nerves in Menidia.

Electrical or mechanical stimulation of the peripheral cut end of the fifth nerve causes movements of the mouth. Sectioning the fifth interferes with the rhythmical activity of the mouth parts that are seen during respiratory movements. Stimulation of the seventh produces closure and opening of the spiracles, and also mouth movements. Sectioning the seventh stops these movements. Stimulation of the peripheral cut end of the ninth causes the dropping or expiratory position of the first gill arch, while lesion of this nerve abolishes its activity. Stimulation of the first to the fourth vagus roots brings about the characteristic inspiratory phases of the second to the fifth gill arches, while cutting these respective roots stops their movements. We see, therefore, that the motor nerves concerned in respiration are the fifth, seventh, ninth, and tenth nerves, of which the fifth is the least important and the tenth the most important. This is because the aeration of the blood is effected if only the last four gill arches are active, all other parts ceasing. On the other hand, the animal may live for an indefinite time, if the spiracle and first gill arches continue their rhythm, all other parts of the respiratory apparatus discontinuing. Attention will be directed mainly to the connections of the seventh, ninth, and tenth nerves in the medulla, as these are the only ones that specially concern us.

From a study of Johnston's[2] article on the brain of Acipenser, and from the results of my experiments and dissections, I judge that the description and histology of the lobes of the medulla of Acipenser and those of the skate are not only homologous but also analogous. Inasmuch as we possess no histological study of the brain of the

[1] Herrick, C. L.: Journal of comparative neurology, 1899.

[2] Johnston, J. B.: Zoologische Jahrbücher, Abtheilung für Anatomie und Ontogenie, 1901, xv, p. 59.

skate, and as it is desirable to know more of the structure of the lobes of its medulla, I think it quite permissible to assume that Johnston's description of the lobes of the medulla of Acipenser may answer also for those of the medulla in the skate. I believe that future work will prove them very much alike in function.

The seventh nerve has extensive connections with the medulla. First, through its dorso-lateral line roots which extend into the lobus lineæ lateralis, and second, through its ventral roots that enter the tuberculum acusticum. Moreover, the anterior division of the lobus vagi consists chiefly of root fibres for the dorsal seventh. Then too the anterior portion of the fasciculus longitudinalis posterior gives off motor fibres which leave laterally to form the motor seventh, at about the level of the lobus lineæ lateralis. Therefore injury to different areas of most of the lobes of the medulla, as will be shown, easily stops or inhibits the movements of the spiracles. The sensory nucleus of the ninth is situated in the lobus vagi, but the fasciculus longitudinalis posterior gives origin to the motor or ventral ninth root. The tenth nerve also has lateral line roots in the tuberculum acusticum, while the posterior region of the lobus vagi receives its sensory roots. In addition to these stations the fasciculus longitudinalis posterior contains numerous vagus roots derived from the motor vagus nuclei situated ventro-mesally. These make their exit, as can be clearly seen, at the side of the medulla. The tenth nerve covers thus a definite but wide area of the medulla. It is directly and indirectly influenced by lesions especially in the posterior part of the medulla and the lobus vagi. We learn from the above description that the lobus vagi embodies the sensory nuclei of the three principal respiratory nerves; it is therefore of special interest to us. The lobus vagi moreover gives rise to the secondary vagus tract, described by Johnston. At the anterior limit of the medulla in contact with the cephalo-ventral surface of the tuberculum acusticum, and at the junction with the cerebellum, lies a group of nerve cells known as the Rinden-Knoten, or the secondary vagus tract nucleus. At the posterior limit of the medulla are two other groups of nerve cells, the nucleus funiculi and the commissura-infima-Halleri. The secondary vagus tract extends from the Rinden-Knoten posteriorly through the tuberculum acusticum and commissura-infima-Halleri to reach the lateral tract in the cord. The importance of this tract I believe lies in the fact, that it acts as an intermediate agent between the vagus and other centres.

Respiratory movements. — The respiratory phases in the skate consist of rhythmical co-ordinated movements for the passage of water into and out of the gill chambers. During the inspiratory phase, the spiracles (*sp.* Fig. 2) and mouth (*m*) open, the arches (1*a*–5*a*) are elevated, permitting the entrance of water, and the gill slits (1*s*–5*s*, Fig. 1) close the openings to the gill chambers. During the expiratory phase which follows, the spiracles and mouth close, the arches drop, and the gill slits open for the exit of water. Occasionally there is a gasping movement consisting of a forcible closure of the gill slits and dropping of the gill arches. During this interval, water and waste matter are expelled through the mouth. In a definite interval of time the respiratory movements may greatly vary, from twenty to seventy or more inspirations per minute. The variation is influenced by depth from the surface, irritation, or internal conditions.

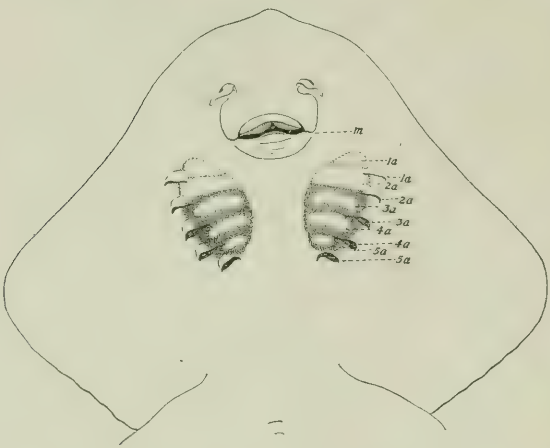

FIGURE 1. — Ventral view of the skate, about half natural size. The outline of the first two gill arches are seen during the inspiratory, the last three in the expiratory phase. *m*, the mouth; 1*a* to 5*a*, outline of first to fifth gill arch; 1*s* to 5*s*, first to fifth gill slits.

EXPERIMENTAL METHODS.

The skate was kept in position on a board by means of a piece of fish netting, and sea-water was passed constantly from a reservoir through a rubber tube into the mouth. The fish remained perfectly quiet for hours, and even days. In this way, or by placing the board with the tube and fish in an aquarium, artificial respiration could be kept up for days. When ether was used, another tube connected with a reservoir of seawater and ether was placed in the mouth. By means of a T-tube joined to the rubber tube and the ether reservoir, ether vapor, ether water, or both could be made

to enter the fish's mouth. The respiratory movements can be seen either from the fish's dorsal (Fig. 2) or from its ventral (Fig. 1) side. If the dorsal side is up, the opening and closing of the spiracles, and the rising and falling of the gill arches can be followed. With the ventral side up, not only the changes in the movements of the mouth, gill arches, and gill slits are seen, but also variations in the heart's action can be studied. The skate, therefore, is an excellent object for the study of changes in the respiratory, as well as the heart's movements. I therefore employed it in my investigation on the effects of intravenous injections of solutions on the central nervous system, with special regard to the heart and respiratory movements. This paper is about to be published. The brain was exposed either from the dorsal side by removing the overlying cartilage, or from the ventral side by cutting through the mucous lining and the roof of the mouth, with scarcely any loss of blood. The effects on respiration, of lesion of any part of the brain, are instantly noticed.

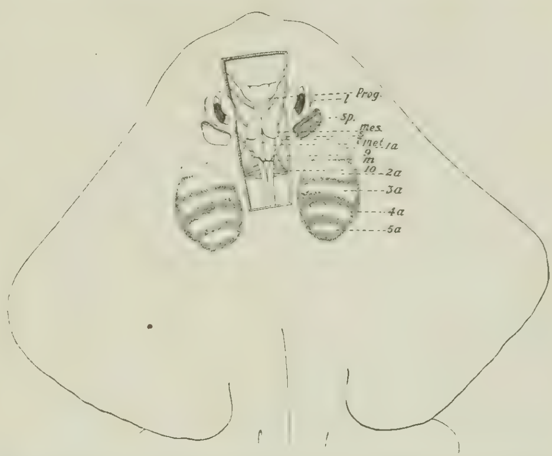

FIGURE 2. — Dorsal view of the skate, showing the outline of the first two gill arches in the expiratory, the last three in the inspiratory phase. The dorsal view of the brain is exposed, and the right spiracle represented closed, the left open. 1*a* to 5*a*, first to fifth gill arches; 5, 7, 9, 10, corresponding cranial nerves; *sp*, spiracle; *e*, eye; *Pros.*, cerebrum; *mes*, midbrain; *met*, cerebellum; *m*, medulla.

Electrical and mechanical stimulations, incisions with small or large scalpels, punctures with needles, or destruction of tissue with red hot needles were employed for different purposes. Stimulation with fine electrodes and threshold value of induction shocks, or pressure with pointed wads of cotton at different points of the medulla in a normal skate, or in one in which the respiration had stopped, or the movements had been inhibited, gave interesting results; but they alone cannot be considered reliable for localization of function, because it is

not definitely known what cells or fibres are acted on by the currents or pressures, both of which may spread wide of their mark. Moreover, the method of sectioning with the scalpel, across several regions of nervous tissue, where there is danger of injuring neighboring areas that may have an influence upon the experiment, is crude and dangerous, unless the function of the entire field sectioned is thoroughly understood, and it is known either that the neighboring parts bear no relation to the parts under investigation, or just how the destruction of those parts would influence the experiment. Before using the scalpel, to determine the effect of removing or separating certain areas, their localities were carefully studied and experimented on, so as to ascertain all possible effects that may arise from a planned lesion, and to be able to interpret the results correctly. After the operation, the animals were observed in an aquarium or on the operating board. They were kept alive by artificial respiration as long as possible. Later the brains were hardened in formole, and the postmortem made. More than sixty skates were experimented upon.

Shock Effects.

Shock effects follow any injury or strong electrical or mechanical stimulations of the different regions of the central nervous system. The effect produces an inhibition of all or of parts of the respiratory movements. The inhibition of the movements of the spiracles is most readily effected, and its duration is always prolonged, lasting many hours or days. The parts inhibited are not, as is believed, always posterior to the place of injury, but may be either anterior or posterior, or both anterior and posterior to the lesion. The nervous tissue anterior to the medulla moreover does not seem capable of preventing the shock, since the inhibition, at least upon the respiratory mechanism, occurs whether the parts in front of the medulla have been removed before the operations on the medulla are performed or not.

Attempts to avoid shock effects were not entirely successful. It was found that cocaine applied locally reduced the time of inhibition, but it greatly depressed the respiratory movements. Ether used carefully while operating, and during the shock, gave more satisfactory results, although it did not entirely prevent inhibition of certain parts of the respiratory apparatus. Lesions made with fine red hot needles

proved less injurious than those made with the scalpel. Intravenous injections of normal salt solution, or of salt solution with a very small percentage of calcium and potassium at least served to shorten the duration of shock. Electrical or mechanical stimulations by pressure or lesion to other parts of the central nervous system during inhibition were of apparent benefit in removing the shock. Much depended upon the condition of the fish. Fish kept in the aquarium for several days would prove more sensitive than those fresh from the sea. Sometimes one gill arch only will begin to move a few minutes after the operation, then other gill arches, or one or both of the spiracles may begin, but perhaps hours or days later. In speaking, therefore, of the duration of shock, I mean the time that elapsed between the operation and first sign of activity of any of the uninjured parts of the respiratory mechanism. Two operations, performed apparently in every respect alike, may cause in one case only slight shock effects of a few minutes' duration, in another case severe and prolonged effects that may last days, or even permanently.

Different levels on the antero-posterior axis of the medulla (Fig. 3) are lettered from *a* to *i*, as an aid in describing the regions operated upon. Many experiments practically alike were carried out, and the general results of such experiments are recorded in the table or text.

Sections Anterior and Posterior to the Medulla.

a. In order to remove the cerebellum from the medulla, to which it is attached by means of the choroid membrane, and to prevent loss of blood and injury to the medulla, it was found necessary to tie a ligature around the base of the cerebellum. In doing so the choroid vessels are tied, and the cerebellum is separated from the mid-brain and the medulla. A shock lasting usually fifteen minutes follows, during which time all respiratory movements cease. At the end of that time the normal rhythm of all parts began again.

b. The medulla was entirely cut off from all the parts of the brain anterior to it, by a transverse section, so that no part of the medulla was destroyed. After a shock inhibiting all respiratory activity for about an hour, the spiracles and, later, the gills began to move again.

c. The medulla was severed from the cord without cutting nerves that emerge from it. For about fifteen minutes following the section, there were no breathing movements visible, then the spiracles and gills slowly began their movements again.

These experiments prove conclusively that the co-ordinated rhythmical activity of the respiratory mechanism is independent of parts of the central nervous system outside the medulla.

Experiments on Special Nuclei.

a. **Rinden-Knoten or nuclei of secondary vagus tract.** — (Described on page 241.) It was of interest to know if this conspicuous nucleus exerted any influence over the gill arches or spiracles. The anterior region of the medulla was gradually destroyed. Beginning at the cephalic end, the middle portion, and then the lateral parts, were burnt with hot needles without any loss to the respiratory action. That is, when the region anterior to (*a*) Fig. 3 was severed from the rest of the medulla, there followed an inhibition of respiration for about twenty minutes, after which time the respiration was again resumed.

b. **The nucleus funiculi and the commissura-infima-Halleri.** — These are situated at the other end of the medulla. This region was completely destroyed by burning. The last two gills ceased their rhythm for about fifteen minutes, and then began again in time with the other parts. This region, corresponding to the area so near to Flouren's respiratory centre, lies outside of the region governing co-ordinated rhythmical respiratory movements.

Medisection of the Medulla.

The next problem was to ascertain the effect of dividing the medulla along the median suture into two lateral halves (Fig. 3). In order to see the whole extent of the suture, the cerebellum was first removed, and ether carefully introduced with the sea-water during the operation. The ether first acts as a stimulant, increasing the rhythm of respiration; but if continued for some time, it retards the activity. To be effective, it must be most carefully used. If with a fine hot needle a cut is made in the median suture along the whole extent of the fourth ventricle, the respiratory movements may all cease for ten minutes or more. As a rule, the gill arches of both sides then resume their activity, though it may happen that the arches of only one side move, and later those of the opposite side begin, or only the last ones of both, or of one side begin, and several hours later the remaining arches join them in their rhythm. Occasionally both spiracles move in the same rhythm with the gill arches, but more

often one spiracle begins to move with the arches of its side, which may be in a rhythm very different from those of the opposite side. If more ether is now given, the spiracle of the opposite side may gradually move. Then both spiracles keep time with the gill arches of one side, or the spiracles and gill arches of the respective sides may have their own rhythm. There may therefore occasionally be no co-ordination of rhythm among the parts of the respiratory organs. Should the cut, however, be made to one side of the suture, the respiratory mechanism of the injured side and the spiracle of the uninjured side will instantly be interrupted and remain inactive for hours and even days. In this case it is certain that the spiracle of the uninjured side is inhibited. The inhibition may last several days, after which it may pass off, and the spiracle then resumes its movements with those of the gill arches.

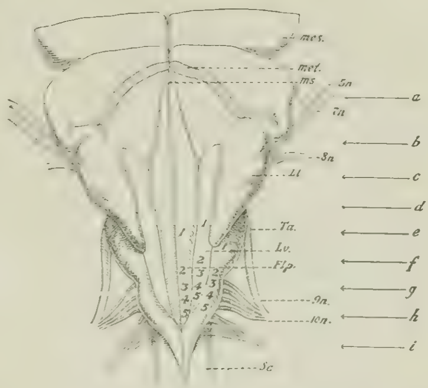

FIGURE 3. — Dorsal view of the medulla. The cerebellum removed to expose the fourth ventricle and the lobes of the medulla. The fore part of the brain was cut off at the posterior junction with the medulla. *a* to *i*, the levels employed to indicate the regions of the medulla, as a guide to the localization of function; 1 to 5 indicating where injury abolishes the function of the corresponding gill arches; *mes*, posterior limit of the midbrain; *met*, cut edge of the cerebellum; *ms*, median suture; 5*n* to 10*n*, corresponding cranial nerves; *Ll*, lobus lineæ lateralis; *Ta*, tuberculum acusticum; *Lv*, lobus vagi; *Flp*, funiculus longitudinalis posterior; *Sc*, spinal cord.

It became evident from the results of median section of the medulla that the centres for the nervous respiratory mechanism in the skate were bilateral, each half controlling the movements of its respective side.

Experiments on the Lobes of the Medulla.

It was of interest to ascertain the effect on the respiratory movements produced by lesion in the different regions of the lobes so characteristic of the fish's medulla (Fig. 3). Furthermore a means was thus offered for comparing ascertained morphological results with physiological ones. Since the motor nuclei lie nearer to the ventro-

mesial surface, and the sensory nearer the dorsal, distinction must be made between deep and superficial injury. A deep puncture penetrates through the medulla, a superficial one aims to penetrate only through the sensory area. When the cerebellum is removed, the outlines of the lobes are distinctly seen, and they can therefore be satisfactorily experimented on from the dorsal side. Operating from the dorsal side of the medulla is more satisfactory than operating from the ventral side, because there are on the ventral surface no definite landmarks to aid in defining the limits of the lobes other than the median suture, the outline at the posterior limit of the medulla, and the exit of some of the nerves. Ventral lesions had to be verified by dorsal ones, and were employed only to ascertain the results of special experiments.

TABLE I.

LESIONS OF THE TUBERCULUM ACUSTICUM (FIG. 3).

Injury to area.	Effect on injured side.	Effect on uninjured side.	Remarks.
a			
b			
c			
d			
e —— ×	no effect 1st arch stopped	no effect " "	superficial puncture. deep "
f —— ×	no effect 2d, or 2d and 3d stopped	" " " "	superficial " deep "
g —— ×	no effect 3d and 4th arches stopped	" " " "	superficial " deep "
h —— ×	no effect 5th arch stopped	" " " "	superficial " deep "

Lesions of the tuberculum acusticum and lobus lineæ lateralis.— (*a*) Superficial injury to the tuberculum acusticum (Fig. 3) caused only slight inhibitory effects on the operated side. Deep punctures or incisions occasioned injury to the underlying motor nuclei, or fibres of either the ninth or tenth nerve, depending upon the area experimented upon. The effect of a lesion, begun at the level *h* and continued gradually anteriorly to level *e* in the area of the tuberculum

acusticum, was permanent suspension of the function of the fifth, fourth, third, second, and first arches respectively (Table I).

Occasionally the puncture aroused the spiracles and uninjured arches to increased activities of short duration.

The table on page 248 gives only the general results obtained from many experiments.

In all the tables, "no effect" means that temporary suspension of function of some parts may have followed, but no permanent suspension.

(*b*) The effect of a superficial lesion beginning at the posterior limit of the lobus lineæ lateralis and extending anteriorly, is to introduce as a rule more or less prolonged inhibition of the spiracle, seldom of both spiracles and the first gill arches. Deep puncture, especially on a level with *c*, *d*, or *e*, which caused injury to the underlying seventh motor nerve, produced sudden permanent cessation of the spiracle activity, and shock effect to the arches of the injured side.

TABLE II.

Lesions of the Lobus Lineæ Lateralis.

Injury at levels.	Effect on injured side.	Effect on uninjured side.	Remarks.
a ——×	no effect " "	no effect " "	deep lesion. superficial lesion.
b ——×	" " " "	" " " "	deep " superficial "
c ——×	spiracle stopped no effect	" " " "	deep " superficial "
d ——	spiracle stopped	" "	deep "
e			
f			
g			
h			

From the fact that in both the tuberculum acusticum and the lobus lineæ lateralis, the nuclei and fibres of the special respiratory nerves are absent, we should not be surprised to learn that they exerted no direct influence over the respiratory mechanism. The lobes overlie the important motor fibres that pass to the spiracles and gill arches,

therefore deep lesions in their areas cause permanent interruption of those parts of the respiratory activity whose fibres have been injured.

Lesions of the fasciculus longitudinalis posterior. — As a rule the punctures and incisions were deep, for superficial injury caused only temporary suspension of function, which passed away usually before twenty-four hours. A puncture, as in other experiments with a hot needle anywhere in the fasciculus between the levels *a* to *c*, stopped

TABLE III.

LESIONS OF THE FASCICULUS LONGITUDINALIS POSTERIOR.

Injury at levels.	Effect on injured side.	Effect on uninjured side.	Remarks.
a	spiracle stops	no effect	deep lesion.
b	" "	" "	" "
c	" "	" "	spiracle on uninjured side inhibited ½ hour.
d	" "	" "	spiracle on uninjured side inhibited 5 hours or until death.
e	spiracle and 1st arch or spiracle only stops	" "	
f	1st arch only or 1st and 2d stop	" "	
g	3d arch or 3d and 4th stop	" "	prolonged inhibition of spiracle and one or more arches on uninjured side.
h	5th arch stopped	" "	

the movements of the spiracle of the injured side, and often the opposite spiracle was also stopped, but the gill arches were not seriously affected by lesions above the level of *d*. Destruction of the tissue just below the region *d* suspended the rhythm of the first gill arch; at *f*, that of the second gill arch; and at the level of *g* usually the third, and often both third and fourth, while the fifth ceased its activity when the injury was made at the level of *h*. Deep incisions at *g* often stopped all respiratory actions of the injured side for many hours, and often all respiratory movements ceased for about half an hour. It is seen from Table III and Fig. 3 that the spindle-shaped area of the fasciculus longitudinalis posterior is capable of division into distinct regions, each of which controls definite respiratory

organs. These results were obtained before I knew of Johnston's [1] article, from which I learned that the above results are due to the destruction of the motor fibres which originate in the definite areas of the fasciculus, as above indicated.

Lesions of the lobus vagi. — Superficial destruction of the lobus vagi, in a line proceeding from the anterior limit posteriorly, gave evidence of localization of the functions of the 7th, 9th, and 10th sensory areas, as illustrated in Fig. 3 and Table IV. For control, the punctures were made both superficially and deep, and the injury in some instances carried from the posterior limit of the lobus anteriorly. It was seen that lesions between the levels *b* and *d* destroyed the movement of the spiracle of the injured side, the opposite spiracle often, and the arches occasionally, ceasing activity for several minutes. Destruction to the area on a level with *c* usually caused the first arch to stop, but occasionally the spiracle also became inactive, and it once happened that both spiracles and all the arches on the injured side stopped moving for three days, from a deep incision in this region; then the arches again resumed their rhythmic movements. A puncture near *f*, if superficial, usually caused both first and second arches to stop moving; but if the puncture was deep, it might strike a motor fibre or cell of the third gill arch, and then the first three arches would become inactive. Under such circumstances, only the spiracle and the last two arches of the injured, and all the parts of the uninjured side, would keep up the rhythm. A superficial injury at *g* suspends the action of the third arch; if the injury is deep, both the third and the fourth gill arch; while at the level *h* the injury is followed by the interruption of the movements of the fifth arch. Punctures made both at *f* and *h* stopped the second and fifth arches, while the spiracle and first, third, and fourth arches of the injured side continued. A deep puncture at *h* may suspend all respiratory function for thirty minutes, and then all but the fifth arch may begin again. If a motor fibre or nerve cell of a spiracle is destroyed in the fasciculus posterior longitudinalis, so that the spiracle no longer moves, a pressure in the area of the lobus vagi *b* to *d* will reflexly cause the spiracle of the uninjured side to stop moving for ten minutes or more, the arches being not thereby affected. This proves that the sensory centre of the injured spiracle is not destroyed, and that the sensory impulses emanating from the spiracle centre act

[1] Johnston: Zoologische Jahrbücher, Abtheilung für Anatomie und Ontologie, 1901, xv, p. 59.

more strongly reflexly on the spiracle than on any other part. When the respiratory movements are slow and weak, they can be suddenly spurred to violent activity by touching a hot needle to, or holding it near any of the areas in the lobus vagi. The organ controlled by cells of that area will suddenly begin to move most rapidly, and all other parts of the respiratory mechanism follow in the same rhythm; thus the rate of respiratory movements may be set by a spiracle, or by any one of the gill arches.

TABLE IV.

Lesions of the Lobus Vagi.

Injury at level.	Effect on injured side.	Effect on uninjured side.	Remarks.
a			
b	spiracle ceased	no effect	deep lesion.
c	" "	" "	superficial lesion.
d	1st gill arch ceased	" "	" "
e	1st gill arch and spiracle stop	inhibition of spiracle or no effect	inhibition of injured side for one hour; deep lesion.
f	2d and 3d, possibly 1st [1] 2d arch stops [1]	no effect " "	deep lesion. superficial lesion.
g	3d or 4th, or 3d and 4th arches stop	" "	" " deep "
h	5th arch ceases	" "	deep lesion; all respiration may cease for 30 minutes.

[1] In some experiments puncture at *f* suspended the function of the first and second gill arches, the spiracle and last three gill arches occasionally continued moving in a different rhythm.

Effect of Transverse Sections of the Medulla.

Since the areas occupied by the sensory nuclei of the principal respiratory nerves in the lobus vagi can now be mapped out, and the result of lesions to the other lobes of the medulla understood, it remains to test the effects of transverse incisions in the medulla on the respiratory movements. The foregoing experiments enabled us

moreover to determine somewhat whether the phenomena following an injury to a region of the medulla are in part or whole the result of shock.

For these experiments the cerebellum was removed. The cut was either made (a) from the median line to near the edge, or (b) carried entirely across the median suture of the medulla. (a) If a hemisection is made at the level *a* or *b*, the spiracle of the injured side instantly stops moving. If made at *c*, usually both spiracles stop, the one due to injury, the other to reflex inhibition. At *d*, the shock effect stops all respiratory movements for about fifteen minutes, then all but both spiracles continue their rhythm again, one spiracle centre injured, the other inhibited. At *e*, both spiracles and first arches stop entirely for twenty-four hours, but sometimes after twenty-four hours the spiracle and first arch of the injured side are the only parts whose functions have been suspended. This seems to be influenced by the method of operation and the condition of the animal. At *f*, the first and second arches, sometimes also the third, of the injured side cease activity due to injury to the cells or fibres. The spiracle and last two or three gill arches of the injured side as a rule move at the same rate as the parts of the uninjured side. The respiratory centres of the organs of the injured side must have been divided into two divisions, — an anterior, consisting of cells and fibres governing the movements of the spiracle, and, in two instances, the spiracle and first arch; and a posterior division, consisting of the last two or three gill arches. These groups usually move in unison, but each may have an independent rhythm of its own for a short time. Hemisection at the level of *g* usually destroys the action of the three last gills of the injured side, the fibres, or motor or sensory nuclei of one or the other being destroyed by the cut. Moreover, sectioning at this or any other level may be followed by a cessation of movements in all gill arches on the injured side, and of both spiracles for a short time. Then too, the spiracle of the uninjured side after such experiments may be inhibited for days, or until the animal dies. Section at the level *h* destroys the movement of the last arch of the injured side. (b) If a deep section is made entirely across the medulla at the level *a*, inhibition of all respiratory movements for one hour ensues, then all parts continue as before. At the level *b* or *c*, both spiracles cease. If the cut is made at the level of *d*, both spiracles and all the arches may stop. After twenty-four hours the three, four, or five last arches continue to move again. Section at *e* suspends all respiratory action

for several days. At the end of the third day, all but the spiracles and first arches are moving. Section at *f* results in shock effect to all of the respiratory mechanism. At the end of the third day both spiracles and the three last gill arches are active, the spiracles moving occasionally in a different rhythm from that of the last three arches. Here the respiratory mechanism is divided into two sections, each having its own rhythm, more distinctly seen and more complete than when hemisection is made.

In some experiments, section at the level of *f* produced shock that resulted in inhibiting all activity for hours, and even after three days, though all but the first arches were active, the spiracles had not resumed their movements. *The inhibition was anterior to the cut and was true inhibition, since we know from other experiments that injury at this level does not suspend the function of the spiracles.* Sections just posterior to *f* showed, at the end of ten days, that all but the 3d and 4th arches were moving. In such experiments the spiracle and first and second arches moved in rhythm occasionally different from that of the fifth arches.

Sections at *g* may cause the last three or four arches to cease, and the first arches and spiracle to move violently for several hours; again the last three arches may cease moving for days or until the death of the animal. Section at *h* has the effect of stopping the last gill arches.

The results noted in Table V, obtained by hemisection and complete transverse section of the medulla, were, it is seen, as might have been expected, not very different; in the one case, cessation occurred on one side only, in the other on both sides. The reflex inhibition of the spiracle, following injury to its fellow, was most marked. Section at (*f*) or (*g*) divided the lobus vagi and fasciculus so that the centres of the respiratory mechanism would be separated into two divisions, each having for a longer or a shorter time a rhythm of its own.

Medisection followed by Hemisection.

When the hemisection followed a median section, which first separated the medulla into a right and a left half, and then one of those halves into an anterior and a posterior division, results were obtained which corroborated those obtained above. The arches and spiracles of the uninjured side continued their activities, and the anterior division consisting of spiracle, or spiracle and first gill arch, might pos-

TABLE V.

Hemisection of the Medulla.			
Injury at level.	Effect on injured side.	Effect on uninjured side.	Remarks.
a	spiracle stops	no effect	deep incision.
b	" "	" "	" "
c	" "	spiracle stops	" "
d	" "	no effect or spiracle may stop	" "
e	spiracle and first arch stops	no effect or spiracle stops	" "
f	1st and 2d stop	" "	" "
g	3d and 4th, or 3d, 4th, and 5th arches stop	spiracle may stop or no effect	" "
h	5th gill arch stops	no effect	" "
Effect of a Transverse Section of the Medulla.			
	Right side.	Left side.	Remarks.
a	no effect	no effect	deep cut.
b	spiracle stops	spiracle stops	" "
c	" "	" "	" "
d	spiracle or spiracle and 1st arch stop	spiracle or spiracle and 1st arch stop	prolonged inhibition.
e	spiracle and 1st arch stop	spiracle and 1st arch stop	inhibited three days.
f	1st and 2d, or 1st, 2d, and 3d arch stop	1st and 2d, or 1st, 2d, and 3d arch stop	" " "
g	3d and 4th, or 3d, 4th, and 5th arches stop	3d and 4th, or 3d, 4th, and 5th stop	" ten "
h	5th arch stops	5th arch stops	deep incision.
Effect of Medisection followed by Hemisection.			
f	1st and 2d, or 1st, 2d, and 3d arches stop	no effect	spiracle and 4th and 5th, or 3d, 4th, and 5th active.
g	3d and 4th, or 3d, 4th, and 5th arches stop	" "	spiracle and 1st and 5th arches active.

sess a rhythm for a few minutes somewhat different; while the posterior division, consisting of the last two or possibly three gill arches exhibited a rhythm which was unlike either of the other divisions. Again, all three divisions kept the same rate of action part of the time. The posterior division, being in some cases separated by the section, first, from the centres of the seventh and ninth nerves, second from the centres of the opposite side, and third, from the ganglia of at least one of the four ganglia belonging to the tenth, must have been stimulated to action through impulses reaching the arches from the three remaining or the one remaining ganglion of the tenth nerve. The movements of these parts were usually more feeble and of shorter duration than those of the other divisions, but that they had an independent centre was evident, and this centre capable of inaugurating and sustaining rhythmical movements of a part of the respiratory mechanism. Transverse sections destroy not only sensory but motor nuclei, and may cut into motor fibres of neighboring arches. For this reason, the function of more than one arch, or arch and spiracle, may be destroyed, and the injury cause entire suspension of function of certain parts. The shock effect was more severe from hemi or complete cross-section than from puncture with hot needles. The period of inhibition depended upon the vigor of the animal, and upon the method employed, and whether the operation was performed with the aid of an anæsthetic or not.

Conclusions.

1. The respiratory movements in the skate are segmental processes. The relationship of the respiratory organs and their segmental centres is not so obvious as it is in lower forms. The developmental changes of shifting and consolidation have begun to mask the segmental connections of the different parts of the brain. Where development has proceeded a step further, this connection would be demonstrated only with difficulty. The results obtained from the experiments on the respiratory centre in the skate, tend to support Loeb's [1] views as to the segmental character of the respiratory centre.

2. The respiratory centre in the skate occupies definite sensory and motor areas in the medulla. The sensory cells, comprising neurons of the seventh, ninth, and tenth cranial nerves, are situated

[1] Loeb: Physiology of the brain, 1900, p. 144.

in the lobus vagi; whereas motor cells and fibres are ventrad to it, as well as in the fasciculus longitudinalis posterior.

3. Each ganglion, through special fibres and cells, controls the activity of the respiratory muscles with which it is segmentally related and is capable of initiating impulses that produce co-ordinated rhythmical respiratory movements.

4. The medulla may be severed both from the cord and the regions of the brain anterior to it, or divided along its median suture, into two bilateral halves, without impairing the functions of the respiratory centre. Each half is capable of sustaining co-ordinated respiratory movements which part of the time may be different in rhythm on the two sides.

5. The ganglia and consequently the respiratory mechanism can be divided into two or three divisions, each of which may for a time have its own peculiar rhythm, or all of the divisions may continue their activity in the same respiratory phases.

6. Not only may either the spiracle and first gill arch, innervated by the seventh and ninth nerves, or the last four gill arches, innervated by the tenth, when isolated from the rest of the respiratory mechanism by a median and transverse section continue their movements, but all other than the special part of the respiratory centre that controls these divisions may be destroyed, and either the four gill arches or the spiracle and first gill arch will still pursue their co-ordinated respiratory activity.

7. There is no one spot, the destruction of which is followed by permanent cessation of respiratory movements, causing sudden death, provided artificial respiration is maintained until the shock effect passes off.

8. Any lesion to the medulla may cause a shock or inhibition to part or all of the respiratory movements for a shorter or longer interval of time. The part whose function has temporarily been suspended may be innervated by nerve cells lying anterior, posterior, or lateral to the lesion.

9. The shock effects may be shortened or prevented if the animal is vigorous; if an anæsthetic, *e. g.* ether, is carefully administered during the operation; if a solution of 20 c.c. $\frac{5}{8}$ n NaCl containing $\frac{1}{10}$ c.c. $\frac{1}{n}$ $CaCl_2$, and $\frac{1}{10}$ c.c. $\frac{1}{n}$ KCl is injected into the blood immediately after the operation; or if a strong electrical or mechanical stimulus, such as pressure, is applied to a region that will either reflexly or directly stimulate the centre of the inhibited part.

10. The skate illustrates, in its type of respiratory centre, an intermediate stage, between the simple segmental arrangement of the neurons presiding over the co-ordinated respiratory movements found among invertebrates, and the complex, modified, and specialized centres existing in higher vertebrates.

ON THE LOCAL APPLICATION OF SOLUTIONS OF SALINE PURGATIVES TO THE PERITONEAL SURFACES OF THE INTESTINE.

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I. The Production and Suppression of Peristaltic Movements of the Intestine by the Local Application of Saline Solutions.

IN a previous paper[1] I have described the effects of subcutaneous and intravenous injections of purgative salts on the intestines of rabbits, and have shown how the peristalsis produced thereby can be inhibited by similar injections of calcium chloride solution. It was further pointed out that these actions are analogous to the production and suppression of rhythmical contractions in voluntary muscles as described by Loeb.[2] This analogy is still more clearly shown by a series of experiments which I have since made on the local application of these salts to the intestine.

I have found that all those salts which produced increased peristalsis when administered intravenously or subcutaneously, or when introduced into the intestine or stomach, have the same action when applied locally to the peritoneal surface of the intestine. The immediate effect of this is essentially local; only those loops which are moistened by the saline solution are at once set in motion. After a short time, the other loops also become active. Peristalsis thus produced may be entirely inhibited by the local application of a solution of calcium or magnesium chloride. This may be illustrated by the following experiment: —

The intestines of a rabbit under the influence of morphine were exposed. On a small group of coils there were poured about 3 c.c. $\frac{m}{8}$ sodium citrate solution.

[1] MacCallum, J. B.: This journal, 1903, x, p. 101; also Preliminary Report, University of California Publications, Physiology, 1903, i, p. 5.

[2] Loeb, J.: Archiv für die gesammte Physiologie, 1902, xci, p. 248.

Almost immediately (within one minute) the loops became very active. Strong contractions of the muscle coats took place. After a few minutes, the other loops were also set in movement, so that the whole small intestine showed active peristalsis. The citrate solution was then washed off by $\frac{m}{8}$ NaCl solution, and about 3 c.c. $\frac{m}{8}$ $CaCl_2$ solution poured on the loops. The peristaltic movements were promptly suppressed, and the intestine remained quiet. By the further addition of citrate solution, the coils were set in active movement once more, and by the subsequent application of calcium chloride solution again inhibited. This was repeated many times (sixteen), and apparently might have been continued as long as the intestine remained alive.

The same results were obtained by using instead of the sodium citrate solution, a solution of barium chloride, sodium sulphate, fluoride, bromide, iodide, phosphate (Na_3PO_4), oxalate or tartrate. Local application of solutions of any of these salts produces increased peristaltic activity. Solutions of sodium chloride have a very slight action of the same character. On the other hand, the intestinal movements are equally inhibited by calcium chloride and magnesium chloride, while strontium chloride has a similar but less powerful inhibiting action.

In testing those salts with which it was necessary to use dilutions greater than $\frac{m}{8}$, the dilution was made with a neutral fluid consisting of sodium chloride and magnesium chloride. It was found that $\frac{m}{8}$ NaCl solution increased to a slight extent the peristaltic movements. By adding to 10 c.c. $\frac{m}{8}$ NaCl, 0.5 c.c. $\frac{m}{8}$ $MgCl_2$, a fluid was obtained which had apparently neither stimulating nor inhibiting effects. In addition to solutions made up by dilution with this neutral fluid, others were used in which the salt solutions were diluted with distilled water. Practically the same results were obtained in both cases. It was found that 1 c.c. $\frac{m}{320}$ $BaCl_2$ solution applied locally to the intestine is sufficient to cause strong peristaltic movements in a rabbit. This quantity contains about 0.00076 gm. barium chloride. In the case of sodium citrate, the concentration must be considerably greater. No solution of this salt more dilute than $\frac{m}{80}$ is active in a rabbit. Of all the purgative salts, barium chloride is by far the most powerful. If a drop of $\frac{m}{8}$ $BaCl_2$ be placed on the serous surface of an intestinal loop, or if a small area be moistened with this solution by means of a camel's hair brush, the muscle beneath the moistened area will almost immediately contract so that a ring-like constriction of the intestine is formed. This often is so sharply marked that it suggests the effect produced by tying

a ligature around the intestine. This constriction remains for a few moments, and then gradually moves along the loop in the direction of the normal peristalsis. If the solution be injected into the muscle of the intestine at any point with a hypodermic needle, a similar sharp constriction takes place. If also a few drops be injected directly into a branch of the superior mesenteric artery, all that part of the loop supplied by the arterial branch will contract violently. These statements are true also in the case of sodium citrate, fluoride, sulphate, etc.; the action of these salts, however, is less powerful.

It must be added here also that the actual passage of fæces may be produced within an hour by the application of the purgative salts to the serous surfaces of the intestine. This takes place most quickly with barium chloride. It is possible to observe directly through the semi-transparent walls of the intestine the rapid passage of fæcal masses from one loop to another.

The intestines of the rabbit are apparently much more sensitive to the action of sodium citrate and sulphate than are those of the dog or cat. Barium chloride, on the contrary, acts with equal strength in all these animals. In a rabbit, the intestines are always set in active peristaltic movement by contact with $\frac{m}{8}$ sodium citrate solution; and even much more dilute solutions are, as a rule, effective. In a cat, however, it was found that a $\frac{m}{8}$ solution of sodium citrate has practically no effect, while a $\frac{5m}{8}$ solution sets the intestine in active motion. In a dog also $\frac{m}{8}$ sodium citrate solution is usually ineffective. Similarly a $\frac{m}{8}$ sodium sulphate solution is inactive in a dog, while a $\frac{m}{2}$ solution of the same salt starts up distinct peristalsis. In the cat and dog, also, the peristalsis may be inhibited by calcium or magnesium chloride, as shown in the following experiment: The intestines of a cat were exposed in the usual manner, and a $\frac{m}{8}$ solution of sodium citrate was applied to the serous surface of the loops. There was no increased movement. There were then poured on the loops a few cubic centimetres of a mixture of 5 c.c. $\frac{5m}{8}$ sodium citrate and 5 c.c. $\frac{5m}{8}$ $CaCl_2$. The loops remained motionless. After waiting a considerable time (fifteen minutes), a $\frac{5m}{8}$ solution of sodium citrate alone was poured on the intestine. Almost immediately they became very active; and the peristalsis continued until calcium chloride was again applied. The loops then came to a standstill. The difference in susceptibility to the action of citrate which exists between rabbits on the one hand, and dogs and

cats on the other, may be in some way connected with their being herbivorous and carnivorous animals respectively.

The action of sodium citrate, sulphate, fluoride, etc., when applied locally, may be inhibited by the administration of an approximately equal quantity of calcium or magnesium chloride of the same concentration. The counteraction of the effect of barium chloride, however, requires a much greater concentration of calcium. Using equal quantities of the two salts, the action of the barium is usually not inhibited, a fact which I have previously stated. With greater concentrations of the calcium chloride, the antagonistic action, however, is clear. This is shown in the following experiment: Applied locally to the intestine of a rabbit 1 c.c. $\frac{m}{320}$ $BaCl_2$ solution caused active peristaltic movements. The application of 1 c.c. $\frac{m}{320}$ $CaCl_2$ solution exercised no inhibiting effect whatever. The same quantity of $\frac{m}{40}$ $CaCl_2$ was then poured on the loops, and a slight but distinct quieting of the loops took place. The addition of 1 c.c. $\frac{m}{8}$ $CaCl_2$ caused the loops to become entirely motionless. After waiting a considerable time, 1 c.c. $\frac{m}{8}$ $BaCl_2$ was poured on the intestine. Immediately violent peristaltic movements took place. Several c.c. $\frac{m}{8}$ $CaCl_2$ exercised practically no inhibiting influence; while 2 c.c. $\frac{5m}{8}$ $CaCl_2$ solution suppressed the movements entirely for a short time.

The question concerning the exact seat of action of the purgative salts remains still unanswered. Whether, upon being absorbed into the blood, they act on the central nervous system is not known. There is no evidence to show that this is the case. It seems certain, on the other hand, from the experiments here described, that they undoubtedly have a peripheral action either on the peripheral nervous mechanism or on the muscle cells themselves. It is impossible to prove that there is no action on the central nervous system, and at present it seems impossible to prove whether the peripheral action is directly on the nerves or on the muscles. The existence in the walls of the intestine of the ganglionic plexuses of Auerbach and Meissner must be taken into consideration; and with the methods available there seems to be no way of distinguishing the action on these plexuses and the direct action on the muscle cells. The ultimate effect is on the muscles and glands; and the fact that an entirely local ring-shaped constriction can be brought about by the local application of a drop of one of the salt solutions to the surface of the intestine would seem to indicate that only a small group of the

circular muscle fibres themselves is affected. The fact that the nerve plexuses form a continuous network, and are intimately related in their various parts, would suggest that the occurrence of an action on these plexuses confined to so small an area is improbable. The discussion of the exact location of the action is, however, of relatively little importance, as compared with the main facts shown by these experiments, namely, that *it is possible to produce, by the local application of a purgative salt to the serous surface of the intestine, a striking increase in peristalsis, and to suppress these movements by a similar application of solutions of calcium, magnesium, or strontium chloride.*

II. The Production of Increased Secretion of Fluid into the Intestine by the Saline Purgatives.

I have already stated [1] that, following the subcutaneous or intravenous administration of a saline purgative, a distinct increase in the secretion of fluid into the intestine takes place. Although this increase can be directly observed, a series of experiments had been planned by which the relative quantities of fluid secreted into an isolated loop of the intestine, before and after the administration of a saline purgative, might be determined by actual measurements. The experiments were interrupted by the summer vacation, and could not be included in the previous paper. Since then, however, I have been able to make these determinations with the following results.

For these experiments rabbits and dogs were used. A loop of considerable length was tied off, and a cannula inserted in its lower end. In the rabbit, the upper part of the small intestine was used, since in this region of the alimentary canal the secretion of fluid seems to be most active. The upper ligature was placed in each case below the entrance of the common bile duct, while the lower ligature and the cannula were placed from twenty-five to thirty centimetres below. By gently lifting the successive loops, the fluid could be easily drained through the cannula. This is made more easy by placing the animal board at a considerable angle with the table. After the loop was emptied, the cannula was closed by a clamp, and the normal secretion allowed to collect in the loop for a period of ten minutes. The contents were then removed and measured. This was

[1] MacCallum, J. B.: This journal, 1903, x, p. 101.

repeated for successive periods of ten minutes, and an estimate of the normal rate of secretion was thus obtained in each case. The purgative salt was then administered either subcutaneously or locally. In the latter case, the solution was allowed to drop from a pipette upon the loop. Care was taken to have the saline solutions at the body temperature, and approximately isosmotic with the blood of the animal. Ten minutes after the administration of the purgative, the loop was again emptied and the contents measured. This was repeated several times. In each case special care was taken to have no interval between the emptying of the loop and the beginning of the succeeding period of ten minutes. In other words, the loop was always entirely empty at the beginning of each period. The operations were performed under morphine; the rabbits were given 5 c.c. 1 per cent solution of the hydrochlorate subcutaneously, and the dogs received the same dose of morphine, and, if necessary, also ether.

The results of the experiments may be seen from the following reports:

1. Rabbit. Loop 30 cm. long, upper part of small intestine.

Loop contained in beginning[1]	5.0 c.c.
Fluid removed after 1st 10 minutes	0.2 "
" " " 2d " "	0.5 "
2 c.c. $\frac{m}{8}$ $BaCl_2$ injected subcutaneously.	
Fluid removed after 1st 10 minutes following injection . .	4.0 "
" " " 2d " " " " . .	3.4 "
" " " 3d " " " " . .	3.0 "

In this rabbit the increased secretion of fluid was accompanied by extremely active peristaltic movements. The fæces could be seen passing along the loops of the lower part of the intestine with great rapidity. Within thirty minutes after the administration of the salt, the passage of fæces to the outside began. This continued for some time, the fæces becoming constantly softer, until finally they were almost entirely unformed.

As shown by this experiment, and also by the following ones, the action of barium chloride persists for a considerable length of time. The action of sodium citrate is more transitory.

[1] In all these experiments there was no interval between the emptying of the loop and the beginning of the ten minute period which followed. The injections were made as rapidly as possible, and in no case occupied more than a minute.

2. Rabbit. Loop 25 cm. long.

Loop contained in beginning	3.0 c.c.	fluid deeply bile-stained.
After 1st 10 minutes	1.0 "	" " "
" 2d " "	0.8 "	" somewhat lighter in color.

Injected 2 c.c. $\frac{m}{8}$ $BaCl_2$ solution subcutaneously.

After 1st 10 minutes following injection	2.5 c.c.	fluid light yellow.
After 2d 10 minutes following injection	1.6 "	" very light yellow.
After 3d 10 minutes following injection	1.8 "	" almost colorless.
After 4th 10 minutes following injection	1.6 "	" quite colorless.
After 5th 10 minutes following injection	1.0 "	" " "

3. Rabbit. Loop 32 cm. long.

Loop contained in beginning	6.0 c.c.
After 1st 10 minutes	0.4 "
" 2d " "	0.1 "
" 3d " "	0.4 "
Poured 5 c.c. $\frac{m}{8}$ sodium citrate on loop.	
After 1st 10 minutes	6.2 "
" 2d " "	2.0 "

4. Rabbit. Loop 30 cm. long.

Loop contained in beginning	5.0 c.c.
After 1st 10 minutes	1.6 "
" 2d " "	1.0 "
Poured 3 c.c. $\frac{m}{8}$ sodium citrate on loop.	
After 1st 10 minutes	1.6 "
" 2d " "	0.2 "
" 3d " "	0.4 "
" 4th " "	0.4 "
Poured 3 c.c. $\frac{m}{8}$ sodium citrate on loops.	
After 1st 10 minutes	1.4 "

5. Dog. Loop 35 cm. long.

Loop contained no fluid, *i. e.*, none could be drained off.

After 1st 10 minutes	0.0 c.c.
" 2d " "	0.0 "
" 3d " "	0.0 "

Poured 3 c.c. $\frac{m}{8}$ $BaCl_2$ on loop.

After 1st 20 minutes	8.0 c.c.
" 2d " "	0.6 "

Poured $1\frac{1}{2}$ c.c. $\frac{m}{8}$ $BaCl_2$ on loop, just enough to moisten it.

After 1st 20 minutes	3.2 c.c.
" 2d " "	2.5 "

It is evident from the experiments that a marked increase in the fluid contents of the intestine follows the administration of the saline purgatives, barium chloride or sodium citrate. That this increase of fluid is due to an actual secretion and not to a delayed absorption, or to the osmotic effect of the salt, is equally clear, since the action takes place most rapidly and most powerfully when the salt is introduced into the blood, or applied to the serous surfaces of the intestine.

Thinking that the disturbance caused by tying off the loop might influence the results, I estimated from the examination of a number of rabbits [1] that the entire small intestine normally contains not more than 10 c.c. of fluid, usually only 5 or 6 c.c. In addition to this quantity of clear fluid there is often found a small amount of semi-fluid food material. To a rabbit in which the intestine seemed comparatively empty, a small dose of barium chloride was administered locally by pouring the solution on the loops. After one hour, the small intestine was removed. It was found to contain 22 c.c. of a clear yellow fluid. In a second rabbit treated in the same way, the intestine contained 34 c.c. of a similar fluid.

The results of further experiments tend to show that calcium applied locally to the serous surface of the intestine exerts an inhibiting action on the secretion of fluid into the intestine. This may be illustrated by the following experiments:

6. Rabbit. Loop 23 cm. long.

Loop contained in the beginning	0.9 c.c.
Fluid secreted during 1st 10 minutes	0.7 "
" " " 2d " "	0.6 "

2 c.c. $\frac{m}{8}$ $CaCl_2$ applied locally.

Fluid secreted during 1st 10 minutes	0.15 "
" " " 2d " "	0.0 "
" " " 3d " "	0.0 "

4 c.c. $\frac{m}{8}$ sodium citrate applied locally.

Fluid secreted during 1st 10 minutes	0.4 "
" " " 2d " "	0.2 "

[1] These rabbits were small, weighing not more than 1200 gms. each.

7. Rabbit. Loop 25 cm. long.

Loop contained in beginning	2.0 c.c.
Fluid secreted during 1st 10 minutes	0.8 "
" " " 2d " "	0.4 "
2 c.c. $\frac{m}{8}$ $CaCl_2$ applied locally to serous surface.	
Fluid secreted during 1st 10 minutes following application .	0.0 "
" " " 2d " " " " .	0.0 "
4 c.c. $\frac{m}{8}$ sodium citrate applied locally.	
Fluid secreted during 1st 10 minutes	0.6 "

8. Rabbit. Loop 22 cm. long.

Loop contained in beginning	2.4 c.c.
Fluid secreted during 1st 10 minutes	1.2 "
" " " 2d " "	1.15 "
3 c.c. $\frac{m}{8}$ $MgCl_2$ applied locally to serous surface.	
Fluid secreted during 1st 10 minutes following application .	0.0 "
" " " 2d " " " " .	0.0 "
" " " 3d " " " " .	0.2 "

Although it is not possible to make a general statement with regard to secretion, it seems probable from these experiments that glandular activity may be influenced by the action of salts in the same way as is muscular activity. The fact that certain salts, *e.g.*, sodium citrate, fluoride, phosphate, etc., are capable of increasing muscular activity, and that certain other salts, *e.g.*, calcium or magnesium chloride have the power of depressing this activity has been clearly shown by Loeb. That peristaltic movements of the intestine may be influenced in the same way by these same salts is shown by the present experiments and by those previously reported. And it seems probable, from the above descriptions, that the relations of these salts to one another have a still more general application, and that their action on the glandular tissues of the intestine is entirely similar to that on the voluntary and smooth muscle of the body.

III. Conclusions.

1. Solutions of many salts, including those commonly known as saline purgatives, produce increased peristalsis when applied locally to the peritoneal surface of the intestine.

2. When administered in this way, extremely small quantities are effective: 1 c.c. $\frac{m}{320}$ $BaCl_2$ (0.00076 gm.) produces well-marked peri-

staltic movements. A somewhat greater concentration of sodium citrate, sulphate, etc., is necessary to produce the same effect. Local contractions of the intestine are produced almost immediately in the part of the loop to which these salts are applied.

3. It is possible to inhibit these peristaltic movements by the local application of solutions of calcium or magnesium chloride to the peritoneal surface of the intestine.

4. In order to inhibit the activity produced by sodium citrate, sulphate, etc., an approximately equal dose of calcium or magnesium chloride is required. The action of barium chloride, however, is counteracted only by a very much larger dose of calcium or magnesium.

5. As shown by direct measurement, the quantity of fluid secreted in a unit of time into a loop of the intestine isolated from the rest of the intestine by ligatures, is much greater after the subcutaneous or intravenous administration of solutions of the saline purgatives. The local administration of the solutions to the peritoneal surface of the intestine has the same effect.

6. The secretion of fluid into the intestine can be inhibited by the administration of calcium or magnesium chloride.

7. It is, therefore, possible to increase or inhibit the activity of the intestine, both muscular and glandular, by the administration of saline solutions. This is entirely analogous to the production and suppression of muscular twitchings in voluntary muscle described by Loeb.

8. These experiments prove still more clearly a statement which I have already made, that the semifluid character of the fæces produced by the saline purgatives is due primarily, if not exclusively, to an increased secretion of fluid into the intestine.

A STUDY OF THE VARIATIONS IN THE COURSE OF THE NITROGEN, SULPHATE, AND PHOSPHATE EXCRETION, AS OBSERVED IN SHORT PERIODS FOLLOWING A SMALL INCREASE IN THE PROTEID INGESTED.

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CONTENTS.

I. Introduction.

THE following investigation was undertaken with a desire to obtain data regarding the course of the nitrogen, sulphate, and phosphate excretion following a comparatively small increase in the proteid ingested. Reports of the related investigations by Sherman and Hawk,[1] and by Hawk,[2] in which the increase in the ingested proteid was very much larger than in the present case, have already been sent from this laboratory. Owing to the greater difficulty in accurately locating the point of maximum elimination with a small

[1] Sherman and Hawk: This journal, 1900, iv, p. 25.

[2] Hawk: This journal, 1903, x, p. 115.

proteid increase, the authors of the present paper, during a part of their investigation, resorted to the use of much shorter periods for urine collection than were thought necessary in the above-mentioned investigations.

The present investigation was carried out with the counsel of Prof. W. O. Atwater, in whose laboratory at Wesleyan University the work was conducted. The authors wish to express their indebtedness to him, and their thanks for the facilities which enabled them to perform the experiments. They are also indebted to Mr. R. D. Milner for many valuable suggestions, and to Messrs. E. Osterberg, W. R. Frazier, and E. M. Swett, for assistance in carrying out the details of the work.

II. Historical.

A review of the former work conducted along lines similar to those of the present study, will be found in a recent article in this journal.[1] Five of the papers there reviewed are more nearly related to the present experiments than the others, in one particular, *i. e.*, the frequency of the urine collections. Voit,[2] Falck,[3] Tschlenoff,[4] Marès,[5] and Hopkins and Hope [6] made at least a portion of their urine collections at hourly intervals. Voit secured three maxima falling in the seventh, twelfth, and sixteenth hours, respectively. Falck, in an experiment on a dog, which he catheterized hourly, secured three maxima, in the fourth, seventh, and tenth hours. The data relative to these three maxima are, however, not strictly comparable with our results, as in Falck's experiment but one meal was taken during the twenty-four hours. Tschlenoff, with his hourly collections of urine, secured but two maxima, in the fourth and seventh hours ; whereas Marès, using hourly periods, found but a single maximum during twelve hours, and that in the eighth hour. Hopkins and Hope in one experiment show two maxima in eleven hours, the maxima falling in the fifth and ninth hours, respectively. In a second experiment, these same investigators secured three maxima in ten hours, falling in this case in the fourth, seventh, and ninth hours.

[1] Hawk : *Loc. cit.*

[2] Voit : Physiologische-Chemische Untersuchungen, Augsberg, 1857, p. 42.

[3] Falck : Beiträge zur Physiologie, Hygiene, Pharmacologie und Toxicologie, Stutgart, 1875, i.

[4] Tschlenoff : Correspondenz-Blatt für Schweizer Aerzte, 1896, xxvi, p. 65.

[5] Marès : Archives slaves de biologie, 1887, iii.

[6] Hopkins and Hope : Journal of physiology, 1898, xxiii, p. 271.

III. Description of Experiments.

Purpose. — The main points toward which attention was directed were, first, the parallelism of the rates of excretion of nitrogen and sulphates, and the independent course of the phosphate excretion; second, the time interval between the ingestion of extra proteid food and the maximum rate of the nitrogen, sulphate, and phosphate excretion following this ingestion; third, to determine whether the small increased proteid ingestion following a condition of nitrogen equilibrium would in any way alter the course of the excretion from that due to a larger increase; fourth, whether the use of shorter periods for the taking of urine samples would not allow a more accurate tracing of the point of maximum excretion; and fifth, whether the maxima would occupy the same positions and be the same in number as under the larger increased proteid ingestion.

Subjects, daily schedule, etc. — The subjects of the investigation were the authors, two young men in good health and with normal digestion. Neither of them used narcotics or stimulants other than tea and coffee, and only one of them, C., used these ordinarily. At the beginning of the experiments, C. weighed 52.28 kg., and at the end 52.38 kg., having gained 100 gms. H. weighed 60 kg. at the beginning, and 59.70 kg. at the end, having lost 300 gms. Both placed themselves upon the diet at the same time, viz.: at the beginning of the experiments, July 16, 1900, at 6.30 A. M., and continued thereon throughout the investigation, until 6.30 A. M., July 25. The date of the experiments being the middle of July, there were a few very warm days, July 17, being 97° F., but neither of the men seemed to be affected either by the diet or by the work connected with the investigation. The muscular work performed while the experiments were in progress, was such as each of the men was accustomed to do, but was a little more confining and severe in length of hours. They rose at 6 A. M., and reached the laboratory in time to breakfast at 6.30 A. M. The second meal came at 12.30 P. M., and the third at 6.30 P. M. At 10 P. M. they retired. The whole day, from 6.30 A. M. until 9.30 P. M., with the exception of an hour for recreation, just before the third meal, was spent in the work connected with the investigation.

Diet, food analysis, etc. — The diet which, with a single exception to be mentioned, was uniform for the three meals of the day, consisted regularly of the following foods: 100 gms. of soda crackers, 20 gms. of butter, and 550 gms. of whole milk. Water was taken, 300 c.c. at

TABLE I.

FOOD ANALYSIS.

Food.		Water.	Ash.	Fat.	Nitrogen.					Heat of combustion.		
					Per cent.	Grams per meal.	Grams, July 20, breakfast.	Grams per day.	Grams, July 20.	Small calories, per gram.	Large calories, per meal.	Large calories, July 20, breakfast.
		per cent	per cent	per cent								
Milk	Composite[1] (partially dry) . .	5.30	5.270	4.42[2]	3.81	....	....	....	....	5395.0	....	....
	Fresh . .	....	....	4.51	0.540	2.970	2.430	8.910	8.370	609.4	335	274
Butter		8.77	....	....	0.195	0.039	0.039	0.117	0.117	8013.0	160	160
Crackers . . .		7.82	1.848	5.94	1.755	1.755	0.878	5.265	4.388	4197.0	420	210
Beef[3]		7.94	2.385	7.33	13.0	....	5.200	....	5.200	5317.0	....	213
Totals		....	....	....	....	4.764	8.547	14.292	18.075	....	915	857

[1] The composite sample of milk was partially dried for analysis = 14.22% P. D.

[2] Fat was determined in the dried composite sample by extraction, and in the fresh sample by Babcock tests made daily.

[3] Beef was partially dried for analysis = 40.0% P. D.

a time, at three periods during the day, viz., at 9.30 A. M., 3.30 P. M., and 9.30 P. M. The crackers, *beef* (see below), and butter were prepared in sufficient quantity at the beginning of the experiments, and samples taken for analysis. The whole milk used was obtained each day from the same cows, thus assuring uniform composition. The milk was daily tested for fat by means of the Babcock test, and an aliquot portion of each day's supply was taken and mixed with similar portions of the milk of the other days, thus making a composite sample. The fat was determined in the composite sample, after partial drying, by the ordinary extraction method. The nitrogen was determined, by the Kjeldahl method, in each day's fresh milk and also

TABLE II.

ANALYSIS OF FÆCES.

Period.	Subject.	Amount in grams.	Nitrogen.		Heat of combustion per gram.
			per cent	grams	small calories
July 16–20 1st milk period	C.	80.3	3.30	2.65	5487
	H.	128.5	3.16	4.06	6018
July 20 Breakfast Meat period	C.	28.9	3.25	0.94	5678
	H.	26.3	3.95	1.04	5104
July 20–24 2d milk period	C.	72.9	3.34	2.44	5160
	H.	82.6	3.53	2.91	5064

in the composite sample. In Table I (page 272) will be found the results of the analyses of the foods. From this table it will be seen that the diet furnished 4.764 gms. of nitrogen per meal, or 14.292 gms. per day, and yielded 915 large calories of energy per meal, or 2745 large calories per day.

Experimental periods and plan of proteid addition. — The uniform diet was continued for four days, until the elimination of nitrogen was fairly regular, as seen in Table III and Fig. 1 (pages 274 and 275).

On the fifth day, July 20, a relatively small amount of extra nitrogen was introduced into the morning meal. One hundred grams of milk and 50 gms. of crackers were replaced by a nearly isodynamic amount of proteid food in the form of very lean beef. The diet for that one meal was: 50 gms. crackers, 20 gms. butter, 450 gms. milk, and 100 gms. lean beef. The beef furnished 5.20 gms. of nitrogen, and

213 large calories of energy. The total nitrogen of this meal was 8.547 gms., and the energy furnished was 857 large calories. The regular meal furnished 4.764 gms. nitrogen, and 915 large calories of energy. The nitrogen was thus increased 3.783 gms., while the energy supplied was decreased by only 58 large calories. At the following meal, the regular diet was again used, and maintained throughout the investigation.

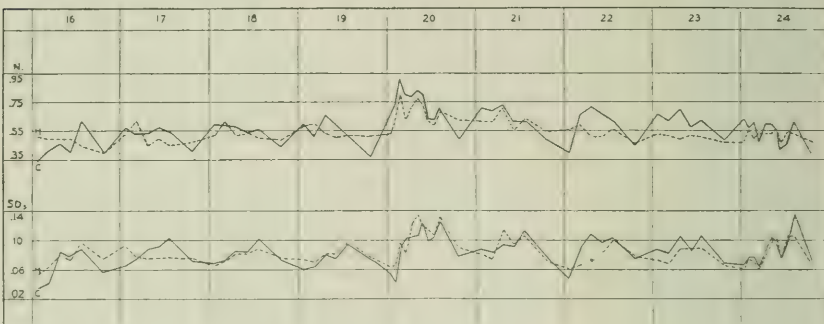

FIGURE 1. — The curves in Figure 1 represent the actual rate of excretion of nitrogen, and sulphates for each subject. The ordinates represent the rate of excretion in grams per hour, the abscissæ, periods of time. The upper pair of curves represent nitrogen, and the lower pair, sulphates. In the curves representing sulphates, the ordinates are drawn to a scale five times greater than in the other curves. C.'s curve is the full line. H.'s curve is the dotted line. * Sample lost.

In this way the experiments were divided into three periods:

I. First milk period: 6.30 A. M., July 16 — 6.30 A. M., July 20 (4 days).

II. Meat period: 6.30 A. M., July 20 — 6.30 A. M., July 21 (1 day).

III. Second milk period: 6.30 A. M., July 21 — 6.30 A. M., July 25 (4 days).

Fæces and urine. — At the commencement of the investigation, and also at the end of each separate period, charcoal, in gelatin capsules, was taken, in order to facilitate the separation of the fæces of one period from those of the period following. The fæces were dried in an air bath at 100° C., carefully weighed, and the nitrogen and heat of combustion determined in the sample of each period.

The urine was analyzed for nitrogen, sulphates, and phosphates. The samples of urine were collected in three hour periods during the day, and a nine hour period at night, excepting on two days, July 20 and 24. During the preliminary days, three hour periods were considered satisfactory, the samples being taken at 9.30 A. M., 12.30 P. M.,

3.30 P. M., 6.30 P. M., 9.30 P. M., and 6.30 A. M. the next morning. On July 20, the day on which the extra proteid was ingested, half periods of one and one-half hours duration were used during the day, between 8.00 A. M. and 9.30 P. M., in order to secure more exact information in

TABLE III.

ANALYSIS OF COMPOSITE URINE.

Subject.	Date. 1900.	Amount of urine in grams.	Nitrogen.		P_2O_5.		SO_3.	
			per cent	grams	per cent	grams	per cent	grams
C.	July 16	641.5	1.52	9.76	0.241	1.540	0.271	1.740
	" 17	739.0	1.57	11.58	0.299	2.210	0.258	1.910
	" 18	885.0	1.37	12.10	0.276	2.445	0.209	1.847
	" 19	972.5	1.20	11.69	0.253	2.461	0.189	1.840
	" 20	1163.0	1.31	15.28	0.288	3.349	0.199	2.317
	" 21	1011.0	1.42	14.32	0.286	2.890	0.204	2.066
	" 22	975.0	1.34	13.02	0.257	2.509	0.205	1.997
	" 23	1111.0	1.24	13.79	0.248	2.756	0.176	1.957
	" 24	972.0	1.22	11.84	0.248	2.406	0.203	1.976
H.	July 16	857.0	1.24	10.61	0.200	1.720	0.209	1.790
	" 17	682.5	1.71	11.66	0.279	1.905	0.271	1.852
	" 18	691.0	1.77	12.22	0.325	2.248	0.270	1.866
	" 19	761.0	1.66	12.76	0.287	2.205	0.251	1.930
	" 20	873.0	1.77	15.45	0.310	2.700	0.280	2.443
	" 21	734.0	1.89	13.85	0.319	2.340	0.279	2.048
	" 22	711.5	1.66	11.82	0.298	2.120	0.279	1.986
	" 23	656.5	1.78	11.66	0.347	2.275	0.272	1.786
	" 24	737.0	1.62	11.96	0.316	2.330	0.243	1.793

regard to the time interval between the ingestion of proteid food and the maximum rate of excretion following it. Then in order to secure a normal day of half periods to compare with the day of increased proteid ingestion, the short periods were again used on July 24, after the nitrogen excretion had again reached a normal uniform rate.

The urine was placed in a refrigerator for a definite time, to cool to a uniform temperature, after which it was weighed and aliquoted for the composite sample. Analyses were then made of the original sample as soon as possible, and at the end of the day the composite sample

TABLE IV.

URINE VOLUMES.

Subject.	Period.	July 16.	July 17.	July 18.	July 19.	July 20.	July 21.	July 22.	July 23.	July 24.
C.	I	73.0	113.4	120.2	158.6	86.7 95.3	142.2	94.4	167.7	112.2 73.2
	II	88.5	102.9	117.8	123.4	125.3 102.1	161.0	160.5	165.6	88.6 64.4
	III	100.6	106.7	131.0	195.7	104.3 95.3	179.2	175.3	179.7	84.6 71.2
	IV	67.9	112.3	111.5	152.2	93.9 79.2	125.0	165.3	135.7	59.9 45.4
	V	118.4	111.6	131.9	133.3	70.6 75.9	137.5	141.3	152.0	82.3 74.5
	VI	193.2	191.9	272.4	209.7	236.1	266.5	237.3	311.1	216.2
	Total	641.6	738.8	884.8	972.9	1162.9	1011.4	974.1	1111.8	972.5
H.	I	163.8	109.0	97.5	125.0	49.3 65.1	100.0	111.0	84.4	45.7 57.5
	II	146.8	159.7	128.9	128.3	93.0 61.0	107.0	104.6	92.5	80.5 62.8
	III	110.0	71.0	90.2	91.4	64.5 60.3	105.3	79.2	79.1	48.5 39.8
	IV	115.9	70.8	77.9	74.7	49.5 50.0	74.3	80.6	78.3	42.0 41.7
	V	118.0	66.9	77.7	76.8	46.8 48.3	104.0	97.7	81.6	49.6 41.8
	VI	202.6	205.0	218.9	264.9	281.8	243.0	239.2	240.3	227.3
	Total	857.1	682.4	691.1	761.1	869.6	733.6	712.3	656.2	737.2

was analyzed. The heat of combustion was determined in the composite sample.

Analytical methods. — The methods of analysis were those in common use in this laboratory.[1] The nitrogen and phosphate determinations were all duplicated, and the totals checked with the analysis of the composite. The sulphate analyses were not made in duplicate, but were checked with the composite sample as in the other cases.

[1] See SHERMAN and HAWK: *Loc. cit.*; HAWK: *Loc. cit.*

IV. Results.

In Table III, page 275, will be found the data for the analysis of the composite urine samples of each subject, and in Table IV, page 276, are given the urine volumes for each period of the several days. Tables V–IX, inclusive, on pages 279, 281, 283, 284, and 285, respectively, give tabular data regarding the nitrogen, sulphate, and

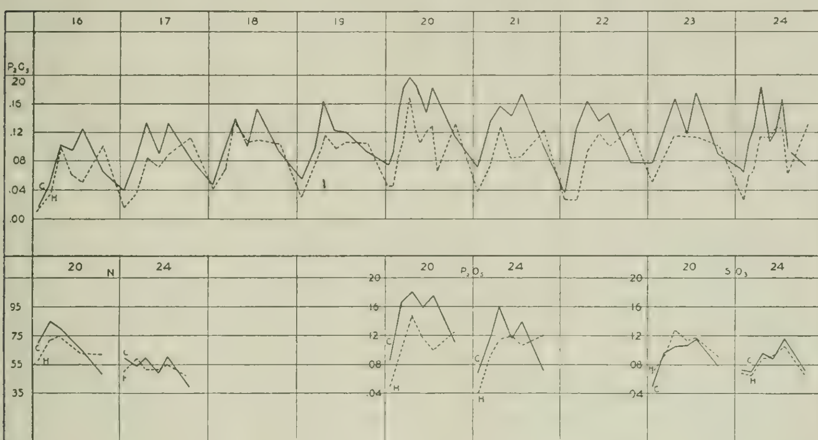

FIGURE 2. — In Fig. 2 the upper pair of curves represent the actual rate of excretion of phosphates for each subject. The ordinates represent the rate of excretion in grams per hour, the abscissæ, periods of time. The one-day curves on the 20th and 24th represent the rate of excretion of nitrogen, sulphates, and phosphates as measured by three hour periods, the same as used on all the other days. The regular curves represent, *on these days only*, periods of one and one-half hours.

The ordinates of the curves for sulphates and phosphates are drawn to a scale five times as great as those of nitrogen.

phosphate excretions, and Table X, page 286, shows the relation between the nitrogen content and heat of combustion of the urine.

In Figs. 1 and 2, pages 274 and 277, the excretion in grams is represented graphically. In these figures the curves are in pairs, in order that the similarity in the course of excretion of the two subjects may be observed.

In Fig. 3, page 280, and Fig. 4, page 282, the graphic representation of the course of excretion for each subject is shown by itself, in order to bring out more clearly the relations between the three substances.

In Fig. 5, page 287, the *average* rate of excretion of the two subjects is represented as in Figs. 3 and 4.

Elimination of nitrogen. — From Table V and Fig. 1, it will be seen that the excretion of nitrogen during the days July 17, 18, and 19, was fairly uniform in both cases, and that this same regularity is again attained to a certain degree on July 22, two days after the proteid ingestion. On the day when the extra proteid food was ingested at the morning meal, the rate of excretion of nitrogen rose rapidly, even during the first short period after the meal. The maximum rate of excretion was reached in the case of both subjects during the third short period, that is, from three to four and one-half hours after ingestion.

Sherman and Hawk (*loc. cit.*) found that the maximum excretion of nitrogen occurred in from six to nine hours after ingestion, whereas Hawk (*loc. cit.*) found the maximum rate of excretion in the case of one subject to occur in from six to nine hours, and in the case of the other subject in from nine to twelve hours. The occurrence of the maximum rate of excretion at an earlier hour was no doubt due in part to the relatively small increase in the ingested proteid, as well as to the use of shorter periods for the collection of the urine.

By an examination of Table V (page 279) or Fig. 1 (page 274), it will be seen that in the case of H., if the collections of urine had been made in three hour periods on the 20th as on the other days, and as in the investigations previously mentioned, the maximum rate of excretion would have fallen in the period from 12.30 P. M. to 3.30 P. M., six to nine hours after the ingestion, instead of between 9.30 and 11.00 A. M. In each case after the maximum was reached, there were two other rises during the day, and at night the rate of excretion was still high. The maxima for each subject occurred in the third, sixth, and tenth periods, or from three to four and one-half hours, seven and one-half to nine hours, and thirteen and one-half to fifteen hours after the ingestion. This seems to agree more nearly with the maxima found by Falck, and also with those of Voit, except that in the latter case the first two maxima are advanced about three hours in each case.

The total amount of nitrogen eliminated in the urine, by C. and H., was almost the same for the two days, the 20th and 21st. During the three following days, the rate of excretion, in the case of C., was much lower than on the 20th, although considerably higher than before the extra proteid ingestion, and it did not regain the normal until the fourth day following the increased excretion. In the case of H., however, the first day after the ingestion was the only one in

TABLE V.

NITROGEN EXCRETION BY PERIODS (IN GRAMS).

Period.		16.	17.	18.	19.	20.[1] 1st half pd.	20.[1] 2d half pd.	21.	22.	23.	24.[1] 1st half pd.	24.[1] 2d half pd.
I	C.	1.03	1.71	1.77	1.79	0.99	1.10	2.10	1.19	2.00	0.95	0.86
6.30 A. M.–9.30 A. M.	H.	1.52	1.58	1.56	1.74	0.78	0.94	1.85	1.67	1.57	0.68	0.83
	Av.	1.27	1.64	1.66	1.76	0.88	1.02	1.97	1.43	1.78	0.81	0.84
II	C.	1.24	1.58	1.75	1.52	1.35	1.20	2.06	1.99	1.87	0.92	0.70
9.30 A. M.–12.30 P. M.	H.	1.44	1.85	1.85	1.80	1.20	0.93	1.83	1.77	1.55	0.95	0.78
	Av.	1.34	1.71	1.80	1.65	1.27	1.06	1.94	1.88	1.71	0.93	0.74
III	C.	1.39	1.59	1.71	1.97	1.17	1.25	2.21	2.14	2.08	0.91	0.87
12.30 P. M.–3.30 P. M.	H.	1.42	1.33	1.54	1.60	1.07	1.16	2.10	1.50	1.45	0.78	0.78
	Av.	1.40	1.46	1.62	1.78	1.12	1.20	2.16	1.82	1.76	0.84	0.82
IV	C.	1.20	1.71	1.63	1.74	1.20	0.95	1.83	1.99	1.70	0.84	0.64
3.30 P. M.–6.30 P. M.	H.	1.49	1.47	1.62	1.51	1.09	0.94	1.65	1.50	1.55	0.83	0.68
	Av.	1.34	1.59	1.62	1.62	1.14	0.94	1.74	1.74	1.62	0.83	0.66
V	C.	1.86	1.62	1.69	1.51	0.94	1.06	1.89	1.86	1.84	0.84	0.92
6.30 P. M.–9.30 P. M.	H.	1.34	1.37	1.48	1.54	0.88	1.02	1.91	1.66	1.50	0.82	0.78
	Av.	1.60	1.49	1.58	1.52	0.91	1.04	1.90	1.76	1.67	0.83	0.85
VI	C.	3.54	3.75	3.97	3.36	4.27		4.31	3.96	4.27	3.44	
9.30 P. M.–6.30 A. M.	H.	3.56	4.23	4.38	4.60	5.61		4.86	4.13	4.19	4.12	
	Av.	3.55	3.99	4.17	3.98	4.94		4.58	4.04	4.23	3.78	
Total . . .	C.	10.24	11.96	12.52	11.92	15.48		14.40	13.13	13.76	11.87	
	H.	10.76	11.83	12.43	12.79	15.61		14.77	12.23	11.81	12.03	
	Av.	10.50	11.89	12.47	12.35	15.54		14.58	12.68	12.78	11.95	

[1] In the 20th and 24th days the long three hour periods are divided into two half periods of one and one-half hour each. The first column of figures gives data for the first half period, and the second column for the second half period.

which excretion remained above normal, and the uniform rate was regained on the 22d. Thus in the case of C., the increase in the excretion of nitrogen after the ingestion of extra proteid food took place, in general, as the previous work from this laboratory has shown; about 80 per cent of the extra nitrogen ingested being excreted during that day, and the maximum rate of excretion occurred shortly after the ingestion, while a marked increase in the rate of excretion was observed for several days. In the case of H., about

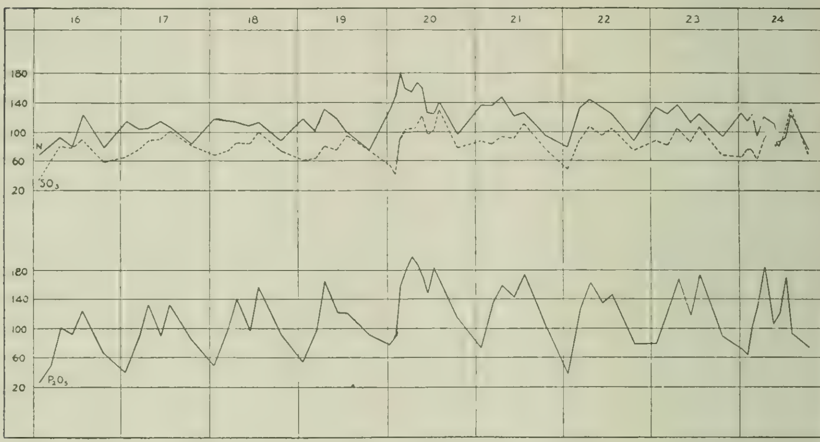

FIGURE 3. — In Figs. 3, 4, and 5, the curves represent variations in the rate of excretion of nitrogen, sulphates, and phosphates expressed in per cents of an assumed average rate of excretion, viz.: 0.5 gram per hour for nitrogen and 0.1 gram per hour for sulphates and phosphates.

The ordinates represent per cent, and the abscissæ, periods of time. The curves representing nitrogen and sulphates are drawn together for comparison, nitrogen being the full line, and sulphates the dotted. The curve for phosphates is the full line at the bottom. Fig. 3 shows the curve for C. Fig. 4 shows the curve for H. Fig. 5 shows the curve representing the average rate of excretion of C. and H.

the same per cent of the extra nitrogen ingested was excreted during that day, but on the day following, viz.: the 21st, and practically during the first half of it, the greater part of the extra nitrogen was eliminated and the rate of excretion fell suddenly to the normal.

By consulting Table IX (page 285), in which the nitrogen balance is given, it will be seen that, during the second and third days after the extra ingestion, C. was metabolizing a larger per cent of the nitrogen ingested than was H., if we may measure the relative amounts of nitrogen metabolized by the amounts excreted in the urine. This fact may account for the difference in time between H. and C. in regain-

TABLE VI.

SO_3 EXCRETION BY PERIODS (IN GRAMS).

Period.		16.	17.	18.	19.	20. 1st half pd.	20. 2d half pd.	21.	22.	23.	24. 1st half pd.	24. 2d half pd.
I	C.	0.103	0.198	0.204	0.184	0.082	0.062	0 266	0.148	0.264	0.099	0.117
6.30 A. M.–9.30 A. M.	H.	0.160	0.279	0.200	0.222	0.096	0.106	0 242	0.183	0.217	0.092	0.109
	Av.	0.131	0.238	0.202	0.203	0.089	0.084	0.254	0.165	0.240	0.095	0.113
II	C.	0.123	0.228	0.220	0.190	0.135	0.154	0.248	0.266	0.244	0.116	0.092
9.30 A. M.–12.30 P. M.	H.	0.190	0.234	0.212	0.210	0.144	0.139	0.218	0.198	0.204	0.105	0.090
	Av.	0.156	0.231	0.216	0.200	0.139	0.146	0.233	0.232	0.224	0.110	0.091
III	C.	0.242	0.267	0.254	0.244	0.156	0.167	0.283	0.326	0.316	0.135	0.154
12.30 P. M.–3.30 P. M.	H.	0.241	0.229	0.246	0.244	0.183	0.204	0.342	....[1]	0.262	0.119	0.148
	Av.	0.241	0.248	0.250	0.244	0.169	0.185	0.313	....	0.289	0.127	0.151
IV	C.	0.237	0.272	0.253	0.228	0.185	0.147	0.274	0.292	0.259	0.149	0.118
3.30 P. M.–6.30 P. M.	H.	0.219	0.228	0.246	0 244	0.179	0.173	0.280	0 244	0.269	0.157	0.114
	Av.	0.228	0.250	0.249	0.236	0.182	0.160	0.277	0.268	0.264	0.153	0.116
V	C.	0.271	0.306	0.304	0.287	0.155	0.197	0.336	0.309	0.319	0.145	0.201
6.30 P. M.–9.30 P. M.	H.	0.294	0.230	0.267	0.298	0.157	0.196	0.325	0.300	0.266	0.159	0.157
	Av.	0.282	0.268	0.285	0.292	0.156	0.196	0.330	0.304	0.292	0.152	0.179
VI	C.	0.535	0.650	0 660	0.673	0.706		0.677	0.663	0.610	0.632	
9.30 P. M.–6.30 A. M.	H.	0.671	0.682	0.677	0.689	0.813		0.644	0.700	0.588	0.599	
	Av.	0.603	0.666	0.668	0.681	0.759		0.660	0.681	0.599	0 615	
Total . . .	C.	1.511	1.921	1.895	1.806	2.147		2.084	2.004	2.012	1.958	
	H.	1.775	1.882	1.845	1.907	2.390		2.144	....	1.806	1.849	
	Av.	1.643	1.901	1.870	1.856	2.268		2.114	....	1.909	1.903	

[1] Sample lost.

ing the normal rate of nitrogen excretion, H. regaining it quickly, near the close of the 21st, and C. not until two days later.

Elimination of sulphates. — Comparing the excretion of sulphates with that of nitrogen for each subject, as shown in Figs. 3, 4, 5 (pages 280, 282, and 287), it will be seen that the rate of excretion of sulphates followed more closely that of nitrogen than did the rate of excretion of phosphates. There is, however, not an exact parallelism. The daily maximum of the nitrogen excretion was usually in the second or third period, while the maximum rate of excretion of sulphates

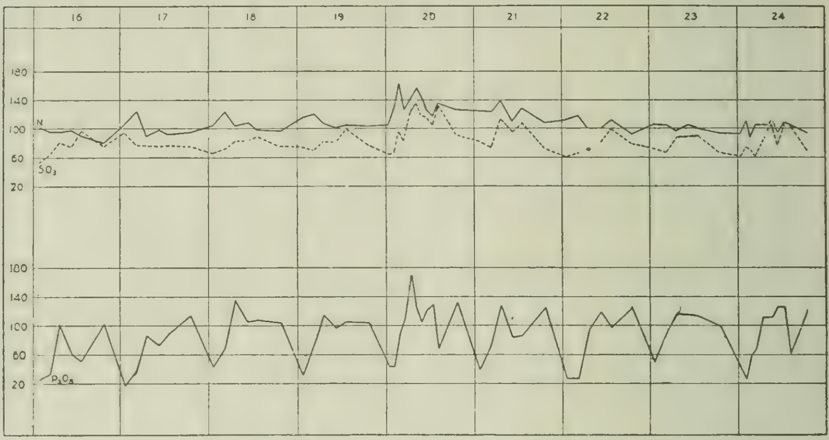

FIGURE 4. — See explanation of Fig. 3. * Sample lost.

usually appeared in the fourth or fifth. Also, while the rate of nitrogen excretion rose immediately after the morning meal, that of the sulphate excretion fell. Thus the maximum rate of sulphate excretion for the day on which the extra proteid food was ingested occurred with C. during the fifth long (three hour) period, and with H. during the third. In the case of H., the rate of excretion of sulphates followed, in general, that of nitrogen on the day following the extra ingestion, and fell to the normal one day later. With C., the rate of excretion of sulphates regained its normal after four days, as was also true of the nitrogen. In this point the rate of excretion of sulphates was contrary to that observed by Garrett[1] in regard to regaining its normal quicker than nitrogen and reaching its maximum earlier.

In Table VII (page 283) the ratio of nitrogen to sulphates is

[1] GARRETT: Journal of physiology, 1898, xxiii, p. 150.

shown. It will be noticed that in the case of C. the ratio is noticeably lower on the day of the increased proteid ingestion than on any of the other days. With H., however, the ratio on that day is an approximate average of the entire period, and the lowest ratio is on the following day.

Elimination of phosphates. — In the investigations already cited, previously carried out in this laboratory, only one rise and fall per

TABLE VII.

Ratio of Nitrogen to Sulphates.

Date, 1900.	C.	H.	Average.
July 16	100 : 14.7	100 : 16.5	100 : 15.4
" 17	100 : 16.0	100 : 15.9	100 : 15.9
" 18	100 : 15.1	100 : 14.8	100 : 15.0
" 19	100 : 15.1	100 : 14.9	100 : 15.0
" 20	100 : 13.8	100 : 15.3	100 : 14.5
" 21	100 : 14.4	100 : 14.5	100 : 14.4
" 22	100 : 15.2	100 : ...[1]	100 : ...[1]
" 23	100 : 14.6	100 : 15.2	100 : 14.9
" 24	100 : 16.4	100 : 15.3	100 : 15.9

[1] Sample lost.

day in the rate of excretion of phosphates was observed in one case, while in the other, in nearly every instance, two distinct rises were recorded.

In the present investigation, however, with both subjects, two such daily maxima were observed, and on the days when short periods were used there were three. These variations in the rate of excretion were more marked in the case of phosphates than with nitrogen or sulphates, and were also more regular. Especially prominent, as shown by the graphic representations, Figs. 3, 4, 5 (pages 280, 282, and 287), was the fall in the rate of excretion during the first morning period immediately following the morning meal. On comparison with the days of short periods, it will be seen that the greater part of this fall in the rate of excretion occurred during the first hour and a half. In most

TABLE VIII.

P_2O_5 EXCRETION BY PERIODS (IN GRAMS).

Period.		16.	17.	18.	19.	20. 1st half pd.	20. 2d half pd.	21.	22.	23.	24. 1st half pd.	24. 2d half pd.
I	C.	0.078	0.120	0.148	0.165	0.116	0.138	0.214	0.118	0.239	0.106	0.096
	H.	0.074	0.053	0.127	0.090	0.065	0.065	0.117	0.082	0.150	0.059	0.039
	Avg.	0.076	0.086	0.137	0.127	0 090	0.101	0.165	0.100	0.194	0.082	0.067
II	C.	0.150	0.270	0.300	0.291	0.235	0.273	0.405	0.385	0.378	0.163	0.193
	H.	0.103	0.113	0.208	0.224	0.132	0.165	0.230	0.082	0.273	0.088	0.109
	Avg.	0.126	0.191	0.254	0.257	0.183	0.219	0.317	0.233	0.325	0.125	0.151
III	C.	0.303	0.395	0.416	0.495	0.299	0.282	0.477	0.490	0.503	0.281	0.203
	H.	0.301	0 248	0.407	0.349	0.253	0.184	0 387	0.276	0.351	0.168	0.168
	Avg.	0 302	0.321	0.411	0.422	0.276	0.233	0.432	0.383	0.427	0.224	0.185
IV	C.	0.279	0.271	0.298	0.367	0.258	0.222	0.426	0.405	0.354	0.160	0.182
	H.	0.180	0.215	0 319	0.294	0.158	0.181	0.248	0.361	0.348	0.168	0.189
	Avg.	0.229	0.243	0.308	0.330	0.208	0.201	0.337	0.383	0.351	0.164	0.185
V	C.	0.377	0.395	0.470	0.364	0 274	0.249	0.521	0.435	0.525	0.254	0.139
	H.	0.151	0.263	0.329	0.315	0.194	0.105	0.259	0.297	0.338	0.187	0.092
	Avg.	0.264	0.329	0.399	0.339	0.234	0.177	0.390	0.366	0.431	0.220	0.115
VI	C.	0.589	0.744	0.826	0.827	1.020		0.902	0.698	0.803	0.656	
	H.	0.906	1 020	0.926	0.936	1.176		1.112	1.121	0.898	1.104	
	Avg.	0.747	0.882	0.876	0.881	1.098		1.007	0.909	0.850	0.880	
Total	C.	1.776	2.195	2.458	2.509	3.366		2.945	2.531	2.802	2.433	
	H.	1.715	1.905	2.316	2.208	2.678		2.459	2.219	2.358	2.371	
	Avg.	1.745	2.050	2 387	2.358	3.022		2.702	2.375	2.580	2.402	

cases, the daily maximum occurred during the third period, but sometimes during the fifth. In the excretion of C., the rate usually fell regularly from the fifth period, and continued during the night, the lowest point occurring during the first period of the morning. With

TABLE IX

NITROGEN BALANCE.

Subject.	Date, 1900.	N in food.	N in fæces.	N in urine.	Gain or loss (+ or −).
C.	July 16	14.29	0.663	10.240	+3.38
	" 17	14.29	0.663	11.955	+1.67
	" 18	14.29	0.663	12.520	+1.11
	" 19	14.29	0.663	11.920	+1.71
	" 20	18.07	1.306	15.480	+1.284
	" 21	14.29	0.518	14.400	−0.628
	" 22	14.29	0.518	13.130	+0 642
	" 23	14.29	0.518	13.760	+0.012
	" 24	14.29	0.518	11.872	+1.900
	Total . . .	132.39	6.030	115.277	+11.083
	Avg. per day	......	....	......	+1.231
H.	July 16	14.29	1.015	10.755	+2.520
	" 17	14.29	1.015	11.825	+1.450
	" 18	14.29	1.015	12.430	+0.845
	" 19	14.29	1.015	12.790	+0.485
	" 20	18.07	1.456	15.610	+1.004
	" 21	14.29	0.624	14.200	−0.534
	" 22	14.29	0.624	12.230	+1.436
	" 23	14.29	0.624	11.810	+1.856
	" 24	14.29	0.624	12.029	+1.637
	Total . . .	132.39	8.012	113.679	+10.699
	Avg. per day	......	....	......	+1.188

TABLE X.

Ratio between Nitrogen and Heat of Combustion of Urine.

Subject.	Date, 1900.	Urine in grams.	Heat of combustion per gram.	Total heat of combustion.	Nitrogen in grams.	Ratio of N : calories.	Calories in excess of urea.[1]
			small calories	large calories			
C.	July 16	641.6	154.6	99.2	9.76	1 : 10.2	46.2
	" 17	738.8	165.0	121.9	11.58	1 : 10.5	59.0
	" 18	884.8	143.4	126.9	12.10	1 : 10.5	61.2
	" 19	972.9	120.8	117.5	11.69	1 : 10.1	54.0
	" 20	1162.9	119.1	138.5	15.28	1 : 9.1	55.5
	" 21	1011.4	136.8	138.4	14.32	1 : 9.7	60.7
	" 22	974.1	133.4	129.9	13.02	1 : 10.0	59.2
	" 23	1111.8	124.5	138.4	13.79	1 : 10.0	63.5
	" 24	972.5	126.5	123.0	11.84	1 : 10.4	58.7
H.	July 16	857.1	131.8	113.0	10.61	1 : 10.7	55.4
	" 17	682.4	163.8	111.8	11.66	1 : 9.6	48.5
	" 18	691.1	171.0	118.2	12.22	1 : 9.7	51.9
	" 19	761.1	160.9	122.4	12.76	1 : 9.6	53.1
	" 20	869.6	148.4	129.0	15.45	1 : 8.4	45.1
	" 21	734.0	190.5	139.8	13.85	1 : 10.1	64.6
	" 22	712.3	181.1	129.0	11.82	1 : 10.9	65.0
	" 23	656.2	178.3	117.0	11.66	1 : 10.0	53.7
	" 24	737.2	163.0	120.1	11.96	1 : 10.1	55.2

[1] See Sherman and Hawk (*loc. cit.*).

H., however, the fall began somewhat later, but reached its point of minimum excretion at the same hour, viz.; during the first period of the day.

The maximum rate of excretion on the day of the increased proteid ingestion was reached during the fifth short period or third long one, one period later than the nitrogen maximum, and earlier than that of the sulphates. The normal was regained with each subject at almost exactly the same time as in the case of the nitrogen.

Nitrogen balance. — Table IX (page 285) gives the nitrogen balance of the experiments. From an examination of the income and outgo

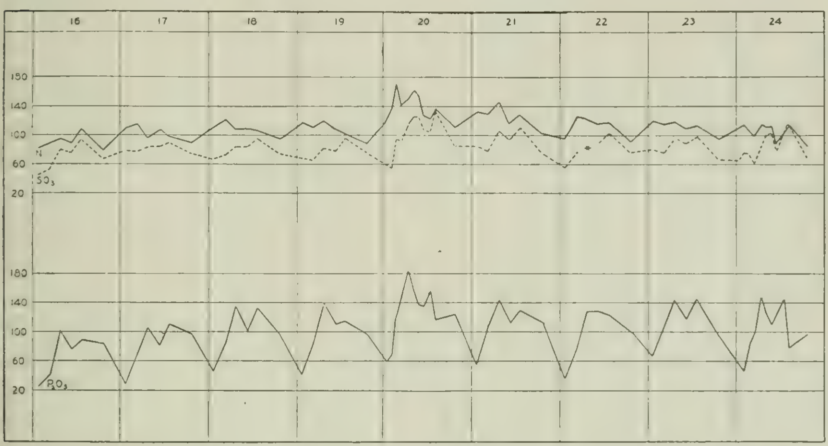

FIGURE 5. — See explanation of Fig. 3. * Sample lost.

of nitrogen, we see that just preceding the ingestion of extra proteid food, both C. and H. were storing nitrogen in about equal amounts. On the three days following the ingestion, C. was approximately in a condition of nitrogen equilibrium, while H. was storing a small quantity. During the whole experiment, however, each subject averaged about the same gain of nitrogen. If now we recall the fact that, in the course of the experiment, H. lost 300 gms. in weight, while C. gained 100 gms., we see that the subjects were not similarly affected by the diet maintained.

Heat of combustion and nitrogen content of urine. — The ratio between the nitrogen content of the urine and the heat of combustion of its unoxidized material was found to vary between 1 : 10.2 on normal days, and 1 : 8.7 on the day of the increased proteid ingestion. These ratios are somewhat higher than others determined in this laboratory

under similar conditions of diet, but in every instance the characteristic minimum ratio, on the day when the extra proteid was eaten, was observed. (Table X, page 286.)

V. Summary.

1. The rate of excretion of nitrogen, measured by three hour periods, showed two maxima daily. The rate rose from the morning meal until midday or a little later, then fell, and rose again about the time of the evening meal. The real maximum rate was usually that of the midday rise, and the minimum rate of excretion took place during the night. Measured by shorter periods, however, three maxima were observed, the real maximum rate coming at a slightly earlier period, and the third rise between this and the evening rise.

2. The rate of excretion of sulphates followed in general a parallel course to that of nitrogen, the main difference being that the minimum rate of excretion was reached after the morning meal, and the maximum late in the afternoon. Frequently three maxima were observed on normal days with three hour periods.

3. The phosphates differed decidedly in their rate of excretion from either the nitrogen or the sulphates. Two very distinct rises were shown each day, and in one instance, that of H. on July 20, an indication of a third rise was seen. The maximum rate of excretion was reached after the midday meal, and was usually the first of the two rises. With one subject, C., the rate of excretion fell from about the fifteenth hour through the night, reaching the minimum during the first period of the morning. With H., the fall in the rate of excretion was later in beginning, but resembled the excretion of C., in reaching the minimum point in the first period of the day.

4. After the ingestion of a small extra amount of proteid food at the morning meal, the rate of excretion of nitrogen reached its maximum within three to four and one-half hours, after which it fell to its normal rate; in one case slowly, after four days, and in the other rapidly, after two days. It would seem that the time required to reach the maximum excretion of nitrogen, after increasing the proteid of a diet, was more or less directly proportional to the amount of proteid ingested, the length of time being greatest when the quantity was large.

5. With each subject the maximum rate of sulphate excretion dif-

fered from that of nitrogen only in reaching its highest point about six hours later.

6. In one subject the ratio of nitrogen to sulphates was lowest on the day of increased proteid ingestion; in the other, on the day after this ingestion.

7. The maximum rate of phosphate excretion due to the increased proteid ingestion fell in a period between those in which the maxima of nitrogen and sulphate occurred.

8. In the case of H., the normal rate of nitrogen and phosphate excretion was regained on the second day following the increased proteid ingestion; the normal rate of sulphate excretion was regained one day later, *i. e.*, three days after the ingestion. With C., the normal rate of excretion was not regained in any case until the fourth day following the increase in proteid food.

9.. The ratio between the heat of combustion of the urine and its nitrogen content was lower on the day of increased proteid ingestion than on normal days.

THE RELATION BETWEEN SOLUTION TENSION, ATOMIC VOLUME, AND THE PHYSIOLOGICAL ACTION OF THE ELEMENTS.

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The Relation between Solution Tension and Physiological Action.

MANY attempts have been made to correlate the physiological action of the elements with their physical or chemical properties, but with only partial success. Thus several writers[1] have established with certain elements and compounds a relationship between physiological action and atomic or molecular weight. Many higher alcohols, for example, are more poisonous than ethyl or methyl alcohol; the sodium salts of iodine are more poisonous, molecule for molecule, than the corresponding but lighter chlorides. Unfortunately, however, this rule of increase of action with an increase of molecular or atomic weight is found to have so many exceptions as to indicate that the relationship is not a simple one. To cite two examples, it is well known that strontium or barium are for most forms of protoplasm less poisonous than the corresponding but lighter metal calcium. Upon the nerve, calcium acts many times as powerfully in inhibiting the action of sodium chloride, as either magnesium or strontium. It is well known besides that the same element will, under different conditions, exhibit very different physiological actions, while its atomic weight does not vary. Ferric salts are more active than ferrous salts. Copper is poisonous for many plants in minute amounts, and, as a rule, far exceeds in action the heavier metal lead.

The attempt to find a connection between physical properties and physiological action was given a great impetus by the ionic hypothe-

[1] Blake: Chemical news, 1887, lv; Proceedings of the Royal Society, London, 1841; Journal of physiology, 1884, v, p. 35; Grützner: Archiv für die gesammte Physiologie, 1893, lii, p. 83.

sis. It has been shown by the work of Kahlenberg and True, Krönig and Paul,[1] and other botanists, and by Loeb and other animal physiologists, that the metals are most active when in an ionic form. Thus, silver as an ion is more poisonous than silver in a complex ion, such as a silver-cyanogen ion, or in combination with albumin. These and many other facts which are well known led to the hypothesis which has been particularly developed by Loeb and the writer in this country, and by Pauli and others abroad, that the elements acted principally by means of their free electrical charges, although some action has been ascribed to the atoms themselves. Loeb and the writer have particularly emphasized the importance of the number of the charges, or valence of the ion in determining its physiological action. The chief difficulty in the way of ascribing action to the number of charges, is the fact that some monovalent ions, like silver, are enormously active, while some trivalent ions, like aluminium, are relatively inert. These facts led me to propose the alternative hypothesis that the movement of the charges was the main cause of the physiological action of ions, and that the different action of two monovalent ions was due primarily to the fact that the movement of the charge varied either as regards its path or speed of rotation about the atom, and I attempted to bring this conception into line with the spectra of the elements.

As this relationship is a difficult one to follow, I have examined a suggestion made two years ago by Professor J. Stieglitz of this university, that the difference between the action of bivalent and trivalent cations and anions described by Loeb and myself might be due not to the difference in number of the charges, so much as to the ease with which the charge is given up from the atom, a trivalent ion like the ferric ion being more efficient physiologically, for the reason that one or more of its charges are given up more easily than are the charges in the bivalent condition. The importance of this factor for other properties of salts had been indicated by Bodländer. That this idea might be fruitful, was indicated by the table of solution tensions of the elements, as given by Nernst[2] and Wilsmore.[3]

[1] Kahlenberg and True: Botanical gazette, 1896, xxii, p. 91; Kahlenberg and Austin: Journal of physical chemistry, 1900, iv, p. 553; Krönig und Paul: Zeitschrift für Hygiene, 1897, xxv, p. 1; Pauli: Hoffmeister's Beiträge zur chemischen Physiologie, 1902, iii, p. 225; Loeb: This journal, 1902, vi, p. 411; Mathews: Science, 1902, xv, pp. 492–498; *Ibid.*, 1903, xvii, p. 436.

[2] Nernst: Theoretische Chemie, 3d ed., 1901, p. 370.

[3] Wilsmore: Zeitschrift für physikalische Chemie, 1901, xxxvi, p. 92.

In a preliminary paper,[1] it has already been indicated that such a relationship between the solution tension of the metal and its poisonous action exists for the motor nerve. In support of such a relationship there was the following reasoning: it is conceivable that the elements act on protoplasm, either by giving up their electric charges or valencies to compounds in the protoplasm, or by taking charges away from these compounds. For example, ferric iron, as is well known, has a strong affinity for a negative charge; it gives up its positive charge and goes very readily into the ferrous state. Now if a ferric ion comes in contact with a molecule which has a negative charge held less firmly than the ion can hold it, the ferric ion will seize that electric charge for itself, and thereby bring about the same kind of a physical or chemical change in the protoplasm as would be produced by an electrolysis.[2] The complex protoplasmic molecule, thus deprived of its charge, would necessarily undergo a rearrangement of equilibrium, in the course of which new compounds would appear, energy be liberated, and protoplasmic movements produced. It is easy to see that the power of any element to produce such a change in protoplasm might depend on its affinity for its positive or negative charge, leaving for the present undetermined the cause of that variation in affinity. Potassium or sodium, for example, having a great affinity for their positive charges, give these up with great difficulty; they would leave the negative charges on the protoplasm unaffected, because sodium and potassium ions, unless they are present in great numbers, have a lower affinity for such charges than has the protoplasm.

There was, therefore, good reason for thinking that the physiological action could thus be measured by the affinity of the element for its charge, those elements, as already said, being relatively indifferent which hold their charges most firmly. This affinity may be measured electrolytically, or it may be computed approximately from the heat of formation of salts. For example, the tension which must exist on the electrodes to separate the elements from aqueous solutions of their salts, may be measured. Such measurements show, in equivalent solutions of completely ionized salts, that it requires a much higher tension to deposit sodium or potassium than to deposit

[1] Mathews: Science, 1903, xvii, p. 436.

[2] This action of a ferric ion may be seen if a little ferric chloride is added to a solution of potassium iodide. The ferric ion takes the negative charge from the iodine.

mercury or silver. The greater the affinity of the element for its ionic charge, obviously the greater voltage will it take to separate that charge from it. This measure is also a measure of the solution tension; that is, of the tendency of the elements to go into solution, and thus to acquire a charge. The following table, taken from Wilsmore,[1] shows the relative solution tensions of different elements for normal ionic solutions. The figures in parentheses have been computed by me from the heat of formation of the salt by the formula $E = \frac{q}{23,110}$ volts, where q is the heat of formation of the salt.

TABLE I.

SOLUTION TENSIONS IN VOLTS FOR NORMAL SOLUTIONS.[2]

K + 2.92	Co − 0.045	I − 0.797
Na + 2.54	Ni − 0.049	Br − 1.270
Rb + (2.54)(?)	Sn < − 0.085	Cl − 1.694
Ba + 2.54	Pb − 0.129	O − 1.396
Sr + 2.49	H − 0.277	Fl (−2.24)
Ca + 2.28	Cu − 0.606	
Li + (2.369)	As < − 0.570	
Mg + 2.26 / 1.214(?)	Bi < − 0.668	
	Sb < − 0.743	
Al + 0.999	Hg − 1.027	
Mn + 0.798	Ag − 1.048	
Zn + 0.493	Pd < − 1.066	
Cd + 0.143	Pt < − 1.140	
Fe″ + 0.063	Au < − 1.356	
Te + 0.045		

An inspection of the foregoing table is sufficient to show that the elements when arranged according to their solution tensions are also arranged approximately according to their poisonous action. Thus we have at one end of the list the extremely poisonous metals, copper, silver, gold, mercury, and platinum; and at the other end, sodium, potassium, strontium, and barium, which are relatively inert. Arsenic,

[1] WILSMORE: Zeitschrift für physikalische Chemie, 1901, xxxvi, p. 92.

[2] While these figures do not measure the absolute affinity of the charge for the atom, except under certain theoretical conditions, they may be used for comparative purposes. The solution tension of oxygen is that of oxygen in acid solutions.

antimony, and bismuth occupy their proper positions as poisonous elements. Beyond gold, we have the negative elements, iodine, bromine, and chlorine, which become less poisonous as their solution tension or affinity for the negative charge increases. The position of hydrogen, which by every other classification is out of place, is here where it should be, near lead and copper. I have already compared this table with the inhibitory and stimulatory effects of salts on the nerve, and showed that the inhibitory effect of the cations and the stimulating action of the anions increased as their solution tensions diminished. A more complete discussion of this relationship will soon be published. At Woods Holl during the summer I was able to get much more readily the data of the physiological efficiency of the salts from a study of the action of salts on the eggs of the fish Fundulus heteroclitus. The eggs of this fish are peculiarly adapted to this work, for the reason that they appear to be easily penetrated by nearly all sorts of ions, and they are quite insensitive to variations in osmotic pressure of the solutions. I determined the least concentration of the various salts which would just prevent the formation of the embryo as a visible line on the egg, that is, a concentration which would kill in about thirty-six hours. The end point, however, is not very sharp. I have confirmed these observations by following the embryos, and determining the concentration which will just prevent pigment formation, or which will kill in about sixty-four hours. The results of the two series were on the whole harmonious. The results here given represent the mean of a large number of experiments, which agreed very well with one another. The eggs were fertilized in sea-water, and then allowed to remain there for from two to four hours, until they were in a 2–8 cell stage. They were then repeatedly washed in fresh and distilled water, and allowed to remain for one hour in a large amount of distilled water, so that the salts already in the egg [1] could dissolve out. About twenty eggs were then transferred with a sieve, so as to carry over little water, to 100 c.c. of the various solutions in finger-bowls. It is important to have the eggs well scattered, not bunched, and to have not more than fifty eggs in this quantity of solution. The room temperature varied from 19° to 26° C. No corrections have been made for temperature, beyond repeating the experiment at different times and taking the means. Observation showed, however, that a small increase in temperature

[1] A determination of chlorine in eggs so washed, showed, however, that a considerable quantity of chlorine remained in the egg.

TABLE II.

Salt.	Dilution.	No. of eggs.	No. of embryos.	Salt.	Dilution.	No. of eggs.	No. of embryos.
1. $AlCl_3$	$\frac{m}{80}$	52	11	$PbC_2(H_3O_2)_2$ (*continued*)	$\frac{m}{13000}$	40	9
"	$\frac{m}{30}$	32	4	"	$\frac{m}{25000}$	21	6
"	$\frac{m}{16}$	41	7	"	$\frac{m}{50000}$	36	28
"	$\frac{m}{12}$	13	5	6. $NiCl_2$	$\frac{m}{400}$	50	5
"	$\frac{m}{9}$	15	0	"	$\frac{m}{200}$	60	1
"	$\frac{m}{6}$	..	0	"	$\frac{m}{50}$	50	4
"	$\frac{m}{4}$	14	0	"	$\frac{m}{24}$	24	0
2. $CuCl_2$	$\frac{m}{6400}$	40	0	"	$\frac{m}{30}$	30	0
"	$\frac{m}{1600}$	25	0	7. $CoCl_2$	$\frac{m}{300}$	20	8
"	$\frac{m}{32000}$	22	0	"	$\frac{m}{200}$	40	18
"	$\frac{m}{30000}$	35	1	"	$\frac{m}{80}$	45	31
"	$\frac{m}{33000}$	60	12	"	$\frac{m}{30}$	20	3
"	$\frac{m}{50000}$	65	23	"	$\frac{m}{24}$	20	1
3. $AgNO_3$	$\frac{m}{10000}$	25	0	"	$\frac{m}{16}$	25	0
"	$\frac{m}{33000}$	22	0	"	$\frac{m}{12}$	22	0
"	$\frac{m}{50000}$	28	0	"	$\frac{m}{8}$	31	0
"	$\frac{m}{100000}$	26	0	8. $ZnSO_4$	$\frac{m}{10000}$	..	many
"	$\frac{m}{100000}$	48	1	"	$\frac{m}{6000}$	..	several
"	$\frac{m}{200000}$	50	3	"	$\frac{m}{2500}$	25	5
"	$\frac{m}{400000}$	60	10	"	$\frac{m}{1650}$	24	0
4. $HgCl_2$	$\frac{m}{25000}$	28	0	9. $ZnCl_2$	$\frac{m}{2400}$	26	4
"	$\frac{m}{33000}$	40	0	"	$\frac{m}{1600}$	24	0
"	$\frac{m}{70000}$	..	0	10. HNO_3	$\frac{m}{10000}$	30	10
"	$\frac{m}{105000}$	70	8	"	$\frac{m}{5000}$	40	6
"	$\frac{m}{210000}$	65	29	"	$\frac{m}{2500}$	28	0
5. $Pb(C_2H_3O_2)_2$	$\frac{m}{5000}$	27	0	11. HCl	$\frac{m}{5000}$	45	1
"	$\frac{m}{10000}$	..	0	"	$\frac{m}{3000}$	27	0
"	$\frac{m}{12000}$	32	0	12. $CdCl_2$	$\frac{m}{300}$	..	0

TABLE II — (*continued*).

Salt.	Dilution.	No. of eggs.	No. of embryos.	Salt.	Dilution.	No. of eggs.	No. of embryos.
$CdCl_2$ (*cont.*)	$\frac{m}{1000}$	..	0	$FeCl_2$ (*cont.*)	$\frac{m}{40}$	65	1
"	$\frac{m}{3000}$	..	0	"	$\frac{m}{24}$	60	4
"	$\frac{m}{10000}$	..	0	"	$\frac{m}{20}$	45	0
"	$\frac{m}{30000}$	45	2	16. $BaCl_2$	$\frac{m}{4}$	25	0
"	$\frac{m}{25000}$	..	0	"	$\frac{m}{5}$	19	8
"	$\frac{m}{50000}$	32	5	"	$\frac{m}{8}$	35	29
"	$\frac{m}{56000}$	62	29	17. $CaCl_2$	$\frac{m}{12}$	42	16
"	$\frac{m}{133000}$	65	45	"	$\frac{m}{8}$	44	3
"	$\frac{m}{400000}$	..	92%	"	$\frac{m}{7}$	36	0
13. $FeCl_3$	$\frac{m}{3000}$	28	0	18. $SrCl_2$	$\frac{m}{3}$	45	20
"	$\frac{m}{6000}$	..	0	"	$\frac{m}{2}$	28	0
"	$\frac{m}{12000}$	22	0	"	$m\,\frac{40}{83}$	11	0
"	$\frac{m}{20000}$	21	3	"	$m\,\frac{3}{8}$	27	0
"	$\frac{m}{60000}$	30	13	"	$m\,\frac{3}{9}$	23	7
14. $MnCl_2$	$\frac{m}{80}$	38	28	19. $AuCl_3$	$\frac{m}{3000}$	19	0
"	$\frac{m}{40}$	29	15	"	$\frac{m}{10000}$	..	0
"	$\frac{m}{26}$	45	15	"	$\frac{m}{20000}$	..	0
"	$\frac{m}{16}$	52	20	"	$\frac{m}{30000}$	..	0
"	$\frac{m}{12}$	35	3	"	$\frac{m}{43000}$	..	0
"	$\frac{m}{8}$	13	0	"	$\frac{m}{75000}$	30	4
"	$\frac{m}{6}$	12	0	"	$\frac{m}{120000}$	18	1
15. $FeCl_2$[1]	$\frac{m}{400}$	..	many	"	$\frac{m}{300000}$	20	16
"	$\frac{m}{200}$	47	39	20. NaCl	$m\,\frac{4}{8}$	18	0
"	$\frac{m}{100}$	..	95%	"	$m\,\frac{3}{8}$	31	1
"	$\frac{m}{80}$	50	12	"	$m\,\frac{3}{9}$	26	5

[1] The ferrous chloride solution was used immediately after making it up. On standing two days or more, its activity increases. The salt was a very good Kahlbaum preparation.

TABLE II — (*concluded*).

Salt.	Dilution.	No. of eggs.	No. of embryos.	Salt.	Dilution.	No. of eggs.	No. of embryos.
21. LiCl	$m\,\frac{32}{80}$	26	0	$MgCl_2$ (*cont.*)	$m\,\frac{22}{80}$	40	0
"	$m\,\frac{3}{8}$	27	0	"	$m\,\frac{20}{80}$	30	1
"	$m\,\frac{2}{8}$	20	0	"	$m\,\frac{18}{80}$	20	3
"	$m\,\frac{28}{80}$	17	1	"	$m\,\frac{1}{2}$	25	0
"	$m\,\frac{21}{80}$	37	12	"	$m\,\frac{1}{4}$	65	8
22. KCl	$m\,\frac{5}{8}$	21	3	"	$m\,\frac{1}{5}$	34	15
"	$m\,\frac{3}{8}$	25	23	"	$m\,\frac{1}{16}$	36	22
"	$m\,\frac{4}{8}$	15	3	"	$m\,\frac{1}{8}$	..	many
"	$m\,\frac{6}{8}$	17	0	"	$m\,\frac{1}{12}$	..	"
23. NH_4Cl	$m\,\frac{3}{4}$	..	0	"	$m\,\frac{1}{16}$	..	"
"	$m\,\frac{4}{8}$	29	3	"	$m\,\frac{1}{20}$	..	"
24. Na_2SO_4	$\frac{m}{4}$	40	0	"	$m\,\frac{1}{2}$	27	0
"	$\frac{m}{5}$	40	2	"	$m\,\frac{15}{40}$	22	0
"	$m\,\frac{18}{100}$	33	2	"	$m\,\frac{13}{40}$	25	0
"	$m\,\frac{3}{10}$	30	2	"	$m\,\frac{8}{40}$	30	2
25. $MgCl_2$	$m\,\frac{26}{80}$	20	0	"	$m\,\frac{9}{40}$	20	0
"	$m\,\frac{24}{80}$	35	0				

increases the poisonous action markedly. I hope next summer to secure the data necessary to calculate the temperature coefficient.

Tables II and III give the concentration in fractions of molecular and in some instances normal solutions, which will just prevent the formation of embryos at a temperature of about 20° C. Most of these salts have been tested several times. The experiments quoted will indicate fairly the character of the results.

It will be seen from the foregoing summary that the chlorides of the metals arrange themselves in nearly the same order, so far as their poisonous action goes, as they are arranged by their solution tension, and we may, therefore, express this fact in the following statement:

The physiological (poisonous) action of any cation varies inversely with its solution tension. — It will be noticed, however, that there is

an enormously greater difference between the poisonous action of mercury and potassium than might have been expected from their difference in solution tension. Furthermore, a difference of one-tenth of a volt near the end of the table determines a greater difference in physiological action than a corresponding difference near the top of the table. This difference is perhaps to be expected, since a difference of one-tenth of a volt when the tension is already low, is greater proportionally than when the tension is high.

Certain exceptions to the general law will be perceived. For example, cadmium is nearly as poisonous as copper, whereas its

TABLE III.

SUMMARY. UNCORRECTED FOR DISSOCIATION.

Salt.	Least poisonous dose.	Salt.	Least poisonous dose.	Salt.	Least poisonous dose.
KCl	$\frac{6}{8}n$	$AlCl_3$	$\frac{1}{3}n$	HCl	$\frac{1}{3000}n$
NH_4Cl	$\frac{5}{8}-\frac{6}{8}n$	$MnCl_2$	$\frac{1}{4}n$	$CuCl_2$	$\frac{1}{15000}n$
NaCl	$\frac{4}{8}n$	$ZnCl_2$	$\frac{1}{800}n$	$AuCl_3$	$\frac{1}{20000}n$
$BaCl_2$	$\frac{4}{8}n$	$CdCl_2$	$\frac{1}{12500}n$	$HgCl_2$	$\frac{1}{50000}n$
$SrCl_2$	$\frac{2}{3}n$	$FeCl_2$	$\frac{1}{10}n$	$AgNO_3$	$\frac{1}{90000}n$
$CaCl_2$	$\frac{2}{7}n$	$CoCl_2$	$\frac{1}{12}n$	$FeCl_3$	$\frac{1}{4000}n$
LiCl	$\frac{1}{4}n$	$NiCl_2$	$\frac{1}{15}n$		
$MgCl_2$	$\frac{14}{50}n$	$Pb(C_2H_3O_2)_2$	$\frac{1}{5000}n$		

solution tension brings it above iron. Ferrous salts are less poisonous than one would expect from the table. Zinc is somewhat out of place, being more poisonous than it should be, and lead, instead of just preceding hydrogen, comes just after it. Gold, again, from the few observations made upon it, was found to be not quite so poisonous as mercury, although it ought to be more poisonous.

These exceptions are to be explained in part by the fact that the different charges on a polyvalent ion are not equivalent, that is, they have different solution tensions, and in part, no doubt, they are to be explained by variations in dissociation. The table just quoted probably gives at the best a mean value for the different charges. Consider, for example, the difference between ferric and ferrous

ions. The ferric ion is more poisonous, equivalent for equivalent, than is hydrogen. This is to be explained by the fact that the third positive charge of the ferric ion leaves the atom with extreme ease. The solution tension for the charge is not, therefore, represented by the figure 0.063, but by a figure more nearly that of copper; in fact, by the figure −0.314 volts, as calculated from the heat of combination of ferric chloride. The ferrous ion is less poisonous, for the reason that the solution tension of the two remaining charges is higher than that given in the table, being approximately 0.076. A ferric ion has, therefore, a much greater affinity for a negative charge than has a ferrous ion. This explanation is confirmed by observing the action of aluminium. The three charges on this element are more nearly of equal value, as aluminium does not form two series of salts, like the ferrous and the ferric salts, and it occupies its proper place in the table of poisonous action.[1] As regards zinc, this element is probably rendered somewhat more poisonous than its position indicates, by the fact that it forms so many free hydrogen ions in its solution. The hydrogen, having a low solution tension, increases the poisonous action of the zinc chloride and sulphate. Cadmium is a marked exception. It is nearly as poisonous as copper, and this is the result obtained also by Kahlenberg and True[2] for seedlings, and by other authors.[3] It is not impossible that the cadmium chloride examined had a trace of silver or mercury in it, but as the difference is so marked, and as it corresponds to the figures of Kahlenberg and True, I am inclined to believe that it is the cadmium itself which is an exception. The explanation of this action is, I believe, the same as in the case of iron. Cadmium has a marked tendency to form double and complex ions, in this respect resembling mercury. The tendency to form such ions has been shown by Abegg and Bodländer[4] to be correlated in the case of any element with a low solution tension. The fact that cadmium forms these double compounds indicates, therefore, that one of its charges has a lower solution tension than the Table shows. The same explanation will hold for lead, but the lead charges are more nearly equal than are those of cadmium or iron. That this

[1] As is shown farther on, aluminium is prevented from exercising its full poisonous action by the protection afforded by the egg envelopes.

[2] KAHLENBERG and TRUE: *Loc. cit.*

[3] KRÖNIG and PAUL: Zeitschrift für Hygiene, 1897, xxv, p. 1.

[4] ABEGG and BOLÄNDER: Zeitschrift für anorganische Chemie, 1899, xx, p. 453.

is at least an explanation of some of these exceptions, is indicated also by a study of the anions.[1]

TABLE IV.

SOLUTION TENSION OF ANIONS.

Cy	[−0.014]	Oxalic	[−0.109]	ClO_3	[−1.138]
OH	−1.157		[−4.105]	CnS	[−0.83] (?)
I	−0.797	Acetic	−1.694(?)	NO_3	−2.229
Br	−1.270		[−4.682]	BrO_3	[−0.727]
Cl	−1.694	Sulphuric	−2.177(?)	IO_3	[−2.536](?)
O	−0.557	Fl	−2.24(?)		

The figures given for the anions show the relative affinity they possess for the positive charge; oxygen, for example, having in an alkaline solution a much lower solution tension as regards the negative charge than iodine or chlorine. The following table gives the least poisonous dose of some salts of sodium and potassium:

TABLE V.

	Least fatal dose.		Least fatal dose.	Anions arranged in the order of poisonous action.	
NaOH[1]	$\frac{1}{200}n$	Na acetate	$\frac{5}{8}n$ (?)	Cy	NO_3
KOH	$\frac{1}{120}n$	$NaNO_3$	$\frac{1}{2}n$	$FeCy_6$	SO_4
$\frac{1}{2}Ba(OH)_2$	$\frac{1}{200}n$	KNO_3	$\frac{6}{8}n$	OH	Br
NaCl	$\frac{1}{2}n$	KCyS	$\frac{3}{8}-\frac{4}{8}n$	Citrate	Acetic
NaBr	$\frac{3}{8}n$	$Na_2C_2O_4$	$\frac{1}{35}n$	C_2O_4	Cl
KBr	$\frac{3}{8}n$	$K_2C_2O_4$	$\frac{1}{25}n$	IO_3	ClO_3
KI	$\frac{3}{8}-\frac{1}{4}n$	$KClO_3$	$\frac{2}{3}n$	BrO_3	
$NaBrO_3$	$\frac{1}{11}n$	$NaHCO_3$	$\frac{1}{4}n$	Fl	
$KBrO_3$	$\frac{1}{10}n$	$CaClO_3$	$\frac{1}{2}n$	HCO_3	
NaFl	$\frac{1}{8}n$	Na_3 citrate	$\frac{3}{400}n$	I	
Na_2SO_4	$\frac{1}{2}n$	K_3FeCy_6	$<\frac{3n}{800}$	CyS	

[1] For the figures relative to the hydrates, I am indebted to my friend, Dr. A. W. GREELEY.

[1] An experimental determination of the decomposition tension of cadmium salts (by ROOT: Journal of physical chemistry, 1903, viii, p. 461) has shown that as the cyanide, oxalate, or phosphate cadmium has a decomposition tension more nearly that of the corresponding copper salts than its solution tension indicates.

It will be seen that for most of these anions the same general rule exists as for the cations, *i. e.*:

The physiological action of the simple anions varies inversely with their solution tension, those of low solution tension being more active than those of high.

The apparent exceptions to this rule are much more numerous than for the cations, and are primarily the fluorides and the complex ions, the oxalates, citrates, nitrates, acetates, bromates, and the iodates.

The exceptions in the case of the complex ions are probably due to the fact that the figures given, which were computed from the heat of formation of the compounds, represent the voltage necessary to break the compound into its elemental form.

These complex ions do not, however, break up electrolytically in this way, but pass into more or less complex fragments. A correction, hence, must be made for each of the complex ions. These corrections are difficult to make with any certainty, but I have indicated the figures for a few of them. It will be perceived, however, that the correction is of such a nature as to bring the compound more nearly in its proper position. For example, the oxalate ion has a solution tension computed from its heat of formation of –4.105 volts. This would indicate that this ion is enormously less poisonous than chlorine, which is the reverse of the truth. When the oxalic ion parts with its charge, it breaks up not into C and O_2, but into CO_2. It takes less energy to bring about this decomposition than is necessary for the complete decomposition of the molecule. To determine this energy, we must subtract from the heat of formation of the oxalate, *i. e.*, 162.35 calories, that of CO_2 or 94.3 calories. With this correction, the solution tension of the oxalate ion should be –0.109. The computation in the next chapter shows that the substitution of this value in the formula stated there gives a result agreeing approximately with the figures found. Similarly, the acetic acid ion has an elemental solution tension of –4.682 volts, which is altogether too high. The acetic acid ion, when it loses its charge, forms CO_2 and C_2H_6. A correction, hence, must be applied to the foregoing figures. If we deduct the heat of formation of both these compounds from that of acetic acid, the resulting solution tension is too small and the ion should be more poisonous than it is. The proper correction for the acetate is accordingly uncertain. Its physiological action would indicate that its solution tension was approximately that of chlorine,

or that the number of acetate ions present is considerably lower than in a chloride solution. More accurate corrections for the organic and complex ions may, perhaps, be obtained from a study of their electrolytic decomposition products.

Fluorine is a marked exception, but I am convinced that the solution tension of this element is certainly lower than iodine. Fluorine, as is well known, is a weak ion. Hydrofluoric acid is decomposed in a $\frac{n}{2}$ solution, at 25°, only to about 13 per cent. It has a strong tendency to form double and complex molecules. Its salts have an alkaline reaction. As a weak dissociation and these other properties are associated generally with a low solution tension, I think it is safe to assume that the solution tension of fluorine is near that which its physiological action indicates, and that it lies between oxygen and iodine. The hydrate tension, *i. e.*, the tension of oxygen in alkaline solutions, is also lower, I believe, than the figures indicate.[1] There are reasons for believing, however, that the two equivalents of the oxygen ion are not equal, but that one of them is lower than the other. This is shown by the fact that the hydrogen of the hydroxyl is only slightly dissociated. McCoy[2] has shown for the carbonate that while one equivalent is nearly as strong as chlorine, the other is very much weaker.

In spite of the exceptions noted, the connection between solution tension and the action of the anions is, I think, unmistakable.

The Relation of Physiological Action of a Salt to its Decomposition Tension.

Having found both for the cations and the anions that their activity varies inversely with the solution tension, I endeavored to find some expression which would enable us to compare salts which have no common ion. In a previous paper the conclusion was drawn, from observations of the action of salts on the nerve, that the physiological action of any salt was due to the sum of the actions of its two ions,[3] and that the two ions had an opposite physiological action. This

[1] Abegg and Bodländer also give reasons for thinking that the solution tension of the oxygen ion is much lower than is indicated. This is shown also by the heat of formation of the oxides.

[2] McCoy: American chemical journal, 1903, xxix, p. 437.

[3] This statement confirms one made by Arrhenius, *i. e.*, "The properties of salt solutions must be capable of representation as the binary sum of the properties of the ions."

conclusion has also been reached independently by Pauli for the precipitating action of salts. We may now state this law in a different form, *i. e., the physiological action of any salt is dependent upon the sum of the solution tensions of its ions* (*both ions being regarded as bearing the same sign*). The sum of the solution tensions constitutes what is known as the decomposition tension. We may say that the physiological action of two salts will be inversely proportional to their decomposition tensions. For example, the decomposition tension of sodium iodide in normal solutions of its ions is equal to that of sodium (2.54 volts), plus that of iodine (0.797 volts), or 3.337 volts. This salt is more powerful in its action upon Fundulus eggs than potassium iodide, with a decomposition tension of 3.715 volts, and this in turn than sodium chloride, with a decomposition tension of 4.234 volts.

It is obvious from these figures and others given in the preceding tables, that any reduction of solution tension in either ion causes a reduction in the decomposition tension of the salt, and an increase in its physiological action. With this obvious fact, I attempted to discover some formula which would enable us to calculate, from the difference in the decomposition tensions of any two salts, what their relative poisonous action would be, and knowing the minimum fatal dose of one to calculate the minimum fatal dose of another. If we arrange the chlorides in a series from potassium to silver, a difference in the concentration of the minimum fatal dose ranging from $\frac{6}{8}n$ to $\frac{1}{90000}n$ is found to exist, corresponding to a difference of 3.968 volts. This indicated that a difference of about 0.14 volt in the decomposition tensions of two successive salts determined a doubling or halving of the poisonous action. Furthermore, it will be found on inspection that this factor of 0.14 volt varies, since it requires more than 0.14 volt difference to cause a halving of the action in the upper part of the table where the solution tension is high. The following empirical formula enables us to make an approximate computation. It lacks a temperature coefficient which my data were not sufficient to give, and also a time factor which may possibly be supplied by farther work.[1]

$$V_a = V_o \frac{1}{2^{\frac{E_a - E_o}{0.14 + 0.03\,E_a}}}$$

[1] Since sending this paper to the printer I have found that the formula may be improved by assuming that a decrease of between one-ninth and one-tenth the value of the decomposition tension will double the poisonous action.

These symbols have the following significance:

V_a = The sought for dilution; *i. e.*, the weakest concentration $(\frac{1}{n})$ of the salt which will kill in a given period (the minimum fatal dose).

V_o = The known dilution of some salt which will kill in the same time.

E_a = Decomposition tension of the salt sought.

E_o = Decomposition tension of the salt known.

For example, to compute the concentration of potassium iodide necessary to kill in twenty-four hours, when one knows that a $\frac{1}{3000}\,n$ solution of hydrochloric acid is just sufficient to kill in that time.

$$V_a = \frac{3000}{2^{\frac{3.717 - 1.417}{0.14 + 0.03\,(3.717)}}} = 0.189\,n$$

By testing a solution of KI, 0.175 n was found to be just sufficient.

Table VI gives the concentration of the solutions (minimum fatal dose) in fractions of normal solutions computed by the above formula, as equivalent in poisonous action to a $\frac{1}{3000}\,n$ hydrochloric acid solution, and the concentrations actually found equivalent by trial. The data were calculated from $V_{\mathrm{HCl}} = 3000$. Time thirty-six hours. The figures representing the concentrations found have been taken from Tables II and IV and corrected for dissociation.

The agreement between the values as computed and found in the above table, while not satisfactory for the metals cobalt, nickel, and ferrous iron, is, on the whole, good. For sodium, barium, magnesium, lithium, strontium, copper, ferric and mercuric chlorides, silver nitrate and potassium iodide, sodium oxalate, and sodium bromate, the values correspond fairly closely. The main quantitative divergencies are found in ferrous iron, aluminium, cobalt, nickel, and sodium, and potassium hydrate. The hydrates occupy, however, the same relative positions to each other, potassium hydrate, both as found and computed, being about one-half as poisonous as sodium hydrate. In a hydrate solution, we must consider oxygen ions as well as hydroxyl. As I am unable to compute the number of free oxygen ions in such a solution, it is impossible to make an accurate computation. When one considers the inaccuracies in the figures for the solution tension, the enormous difference in poisonous action of sodium and mercuric chlorides, the temperature variations, and variations in the eggs, the values found agree, in many instances, astonishingly well with those computed. The agreement in the case of potassium iodide is es-

TABLE VI.

COMPUTED AND FOUND VALUES OF MINIMUM FATAL DOSE IN TERMS OF A NORMAL SOLUTION.

Salt	KCl	NaCl	$BaCl_2$	$MgCl_2$	$CaCl_2$
Computed . .	0.948	0.500	0.500	0.302	0.306
Found	0.507	0.455	0.322	0.610	0.200
Salt	LiCl	$SrCl_2$	$AlCl_3$	$MnCl_2$	$ZnCl_2$
Computed . .	0.367	0.462	0.018	0.0107	0.0044
Found	0.285	0.442	0.133	0.125	0.0012
Salt	$FeCl_2$	$FeCl_3$	$CoCl_2$	$NiCl_2$	$PbCl_2$
Computed . .	0.0012	$0.\overset{3}{0}29$	$0.\overset{3}{0}78$	$0.\overset{3}{0}76$	$0.\overset{3}{0}57$
Found	0.065	$0.\overset{3}{0}25$	0.060	0.053	$0.\overset{3}{0}2$
Salt	$CuCl_2$	$HgCl_2$	$AgNO_3$	NaOH	KOH
Computed . .	$0.\overset{4}{0}87$	$0.\overset{4}{0}13$	$0.\overset{4}{0}11$	0.049(?)	0.092[1](?)
Found	$0.\overset{4}{0}66$	$0.\overset{4}{0}15$	$0.\overset{4}{0}11$	0.005	0.010
Salt	KI	NaI	$K_2C_2O_4$	$Na_2C_2O_4$	$NaBrO_3$
Computed . .	0.189	0.083	0.034	0.020	0.072
Found	0.175	..	0.040	0.028	0.090

[1] The values for the hydrates were computed from the solution tension of oxygen as equal to –0.557. The computation on this basis is arbitrary. If the solution tension of $\overline{OH}$ is used, values still farther removed are obtained. If, however, the figures for the oxygen ion computed from the oxides be substituted, values are obtained which err on the other side. (See discussion.)

pecially good, since in this case we have computed the value of a salt which has no ion in common with hydrochloric acid, upon which the computation was based. The discrepancies of ferrous iron, cobalt, and nickel are unexplained, but may possibly be due to these ions penetrat-

ing the egg membranes with more difficulty, or to the formation of complex ions. As a matter of fact, it could be seen that some iron was precipitated, as the hydrate or basic carbonate (?), on the outside of the eggs, thus reducing the concentration. Also cobalt and nickel, while preventing the formation of an embryo, only in strong doses, killed the embryo when formed in very much weaker doses, giving the impression that these ions got into the egg slowly. With aluminium chloride, while the embryo lived three weeks, and hatched in the solution of $\frac{1}{8}n$ $AlCl_3$, it died within two to three hours after emergence, indicating again that these membranes in some way protected the embryo during embryonic life.

In the experiments of True and Kahlenberg, of Heald, and of Clark, who tested, respectively, the action of salts on the growth of roots of lupinus albus, pisum sativum, zea mais, and the sprouting of mould spores, cobalt and nickel salts come much nearer their true position, being almost as poisonous as copper. This fact indicates that special conditions prevail in the Fundulus egg which render these salts less active. It is to be expected, I think, that such particular variations will be found to exist in different forms of protoplasm. This is a subject to which I hope to return more at length, but mention may be made of Heald's results, which showed that, for nearly all forms of salts and acids, zea mais was more resistant than pisum. We know, besides, from the work of Stewart and others, that the red blood-corpuscles are much more readily penetrated by some salts than by others, and this, no doubt, influences physiological action. There is, however, a practical agreement among observers that cobalt salts are a little less poisonous than nickel salts, and that cadmium is intensely poisonous.

In True and Kahlenberg's work on lupinus it was found that acids were fatal in a dose of about $\frac{n}{6000}$. This is about twice as dilute as the corresponding fatal dose for Fundulus. It is interesting to note that a similar difference was observed for silver, copper, and other metal salts, which were also poisonous in a lower concentration than for Fundulus. On the other hand, a curious exception is seen in the action of salts upon moulds. These forms are enormously resistant to acids, and corresponding with this we find them enormously resistant to metals. Penicillium, for example, was found by Clark[1] to be killed in the spore stage by $\frac{n}{16}$ hydrochloric acid. It required a $\frac{n}{512}$ solution of $Cd(NO_3)_2$; a $\frac{n}{65536}$ of $AgNO_3$; a $\frac{n}{16384}$ solution of $HgCl_2$; and a $\frac{n}{32}$ solution of $Cu(NO_3)_2$ to produce the same result. It was not killed

[1] CLARK: Botanical gazette, 1899, xxviii, p. 289.

in a double normal sodium chloride solution. A curious exception, however, is to be observed in the susceptibility to hydrates, a $\frac{n}{40}$ solution of potassium hydrate being fatal. In this case the hydrate ion (oxygen ion) is more fatal than the hydrogen; whereas for Fundulus and peas, beans, and corn the reverse is the case; while the figures given by Kahlenberg and True and by me for the hydrates are probably too high, owing to the formation of carbon dioxide by the protoplasm and its absorption from the air, the discrepancy is too great to be accounted for in this way. It looks as though there might be two conditions or kinds of protoplasm, one of which is particularly sensitive to changes in the solution tension of the positive ion, the other to changes in the negative ion. A similar relation may perhaps be seen in the case of ferments, like ptyalin, which are quickly inhibited by very small amounts of hydroxyl ions, but require more hydrogen to accomplish the same result. Greeley's reversal of the electrotonic reactions of infusoria and certain tissues by a reversal of the alkalinity of the medium, may also be brought into line. At present, however, these exceptions can only be pointed out and left for future work to clear up.

In spite of the divergences, therefore, the results of the computation are sufficiently close to indicate that the figures for decomposition tension will give us a means of computing physiological action. A better formula can no doubt be found, and with other materials the constants can be determined more accurately. We have at any rate for the first time the means of computing physiological action from one salt to another, with at least approximate accuracy. Mr. McGuigan, in this laboratory, has found that the elements inhibit ferment reactions in the same order, and his results, I think, will give much sharper end-points and truer relationships than any obtained on living cells. If this relation between solution tension and physiological action be substantiated, as I have no doubt it will be, the physiological test may give us a valuable means of determining solution tensions.

My results confirm Bodländer and Abegg that the ClO_3 ion in its sodium combination is somewhat less active than chlorine. For the bromate and iodate, however, there is a marked discrepancy. From my results, the solution tension of these ions (Haftintensität) is low; whereas Bodländer, from their solubilities, considers the solution tensions to be high. There are many indications, it seems to me, that these ions have a small solution tension. But I hope to take this up more in detail later.

The facts already stated show very clearly that both ions are of importance in determining the physiological action of any salt, and that the general rule stated in a previous paper, that the action of any salt is equal to the sum of the actions of its ions is correct. It would seem unnecessary to emphasize this almost self-evident fact, were it not that many physiologists have concluded that the action of the salt is due primarily to the cation, or the anion. Loeb and many other writers, for example, refer to the action of sodium salts as due to the sodium ion. True and Kahlenberg state that the anions are relatively inert; [1] some authors have attempted to show that the cation, hydrogen, is alone active in the action of acids on digestion, although Loeb and Giess refer to the action of the anion as being also of some importance in the action of acids in Fundulus eggs, and Sjöqvist considers it of importance in the action of acids on pepsin. While it is true that there is a far greater quantitative difference in the action of the cations sodium and mercury than is to be observed in the action of most anions, this is due to the fact that there is less variation in the solution tensions of the common anions than in those of the cations.

Sodium chloride, nitrate, sulphate, and acetate act much alike in my opinion, not because the action of the sodium is so dominant as to render immaterial the action of the anion, but because all these anions have nearly the same high solution tension, or because of lessened dissociation, and the formation of complex ions, the total effect in equivalent concentrated solutions is about the same in each. A close examination, however, will show very clearly that even these salts differ one from another, and if the hydrate, in which a relatively low anion is present, be considered it will easily be seen that variation of the anions are as important as variations in the cations. The variation, for example, in solution tension of the anions chlorine and iodine is no greater than that of sodium and magnesium. But every pharmacologist knows that the iodide and chloride of sodium differ in their physiological action. This rule holds also for the action of salts on ferments, as has been shown in the case of the action of various acids on pepsin digestion. As showing the action of the anions, the following experiment is of interest:

[1] KAHLENBERG, in a later paper, however, has modified this conclusion: Journal of physical chemistry, 1901, iv. The explanation for the exceptions he notes in the taste and action of acids and salts is to be found in part, I think, in the action of the anion, as he suggests.

EXPERIMENT XIX.

FUNDULUS EGGS TRANSFERRED TO THE SOLUTIONS AFTER 28 HOURS IN DISTILLED WATER.

Salt.	Concentration. n	Eggs.	Embryos.	Salt.	Concentration. n	Eggs.	Embryos.
NaCl	$\frac{3}{8}$	32	31	$NaBrO_3$ (*cont.*)	$\frac{1}{10}$	15	1
"	$\frac{4}{8}$	41	30	"	$\frac{1}{16}$	39	38
"	$\frac{35}{80}$	26	25	NaFl	$\frac{5}{8}$	14	0
"	$\frac{65}{166}$	46	44	"	$\frac{1}{8}$	21	7
$NaC_2H_3O_2$	$\frac{32}{80}$	26	24	"	$\frac{1}{4}$	8	0
"	$\frac{4}{9}$	26	20	"	$\frac{1}{100}$	12	12
"	$\frac{1}{5}$	21	21	$\frac{1}{2}$ $Na_2C_2O_4$	$\frac{1}{10}$	26	0
"	$\frac{3}{5}$	33	16	"	$\frac{1}{20}$	21	4
"	$\frac{1}{10}$	28	28	"	$\frac{1}{40}$	22	21
$\frac{1}{2}$ Na_2SO_4	$\frac{36}{80}$	36	31	"	$\frac{1}{30}$	33	33
"	$\frac{34}{80}$	37	28	"	$\frac{2}{5}$	30	0
"	$\frac{30}{86}$	28	25	"	$\frac{1}{5}$	32	0
"	$\frac{28}{80}$	30	26	"	$\frac{1}{200}$	19	17
NaBr	$\frac{28}{80}$	36	31	"	$\frac{1}{400}$	20	20
"	$\frac{1}{5}$	23	22	$NaNO_3$	$\frac{5}{8}$	32	17
"	$\frac{1}{100}$	27	27	"	$\frac{4}{8}$	45	33
"	$\frac{30}{80}$	19	16	"	$\frac{3}{8}$	24	21
"	$\frac{32}{80}$	22	17	"	$\frac{2}{8}$	45	44
$NaBrO_3$	$\frac{20}{80}$	15	0	Control	H_2O	29	28
"	$\frac{16}{80}$	28	0				

In this experiment it may readily be seen that small differences exist between such salts as sodium acetate, sodium chloride, and sodium bromide, and a very great difference between the bromate and bromide.

In a previous paper, evidence was given showing that the positive and negative ions have an opposite physiological action on the motor nerve. This fact can be seen readily in the case of Fundulus, unless we assume, as is discussed further on, that the two ions act on each

other. This assumption we will not consider here, but instead assume that the current hypothesis of the independence of the ions is correct. Potassium hydrate is less poisonous than sodium hydrate. Here we have in each case the same number of hydroxyl ions, an ion which is extremely strong chemically and physiologically, and hence might be supposed to mask absolutely the action of the other ion, but this it does not do. Assuming that the ions are independent, this difference of action can only be explained by the fact that potassium has a physiological action opposite to that of the hydroxyl, so that it counterbalances to a certain degree the anion action. All potassium salts are, as a matter of fact, less poisonous for Fundulus than the corresponding sodium salts, as is shown by the following experiment:

EXPERIMENT XXVI.

Salt.	Concentration.	Eggs.	Embryos.	Salt.	Concentration.	Eggs.	Embryos.
$NaNO_3$	$m\,\frac{4}{8}$	39	1	KNO_3	$m\,\frac{4}{8}$	28	12
"	$m\,\frac{35}{80}$	37	1	"	$m\,\frac{5}{8}$	27	10
"	$m\,\frac{30}{80}$	27	1	"	$m\,\frac{3}{8}$	36	16
NaBr	$m\,\frac{40}{83}$	37	0	KBr	$m\,\frac{40}{83}$	28	11
$NaBrO_3$	$\frac{m}{10}$	16	0	$KBrO_3$	$\frac{m}{10}$	28	1
"	$\frac{m}{15}$	31	2	"	$\frac{m}{15}$	25	3
"	$\frac{m}{20}$	25	3	"	$\frac{m}{20}$	22	8
"	$\frac{m}{40}$	27	21	"	$\frac{m}{40}$	15	13
Na oxalate	$\frac{m}{50}$	22	0	K oxalate	$\frac{m}{50}$	22	1
"	$\frac{m}{70}$	19	0	"	$\frac{m}{70}$	19	1
"	$\frac{m}{80}$	31	5	"	$\frac{m}{80}$	23	3
"	$\frac{m}{90}$	29	6	"	$\frac{m}{90}$	51	17

The establishment of the fact that the anions and cations have opposite physiological actions invalidates the computations made by True to determine the relative poisonous action of anions, cations, and undissociated molecules. It is plain that the action of every potassium salt is dependent upon the anion as well as upon the cation. It is hence incorrect to determine the physiological action of an acetate ion from the poisonous action of sodium acetate, and

then to insert this value in a computation for acetic acid. We cannot assume even that the acetic acid ion when associated with hydrogen has the same value as the acetic acid ion when associated with sodium. Sodium and potassium are apparently able to neutralize the action of the anions in their salts much more completely than hydrogen can. The counteracting action of any ion upon another of opposite charge appears to increase with its solution tension. This fact of the counteracting action of the opposite ions, for which other evidence will be presented in my work on nerve, throws possibly a new light also on the sour taste of acids. Kahlenberg suggests that the anions taste sour, as well as the hydrogen ions, in order to explain the fact that acids with weak anions taste sourer than they ought. The explanation of this fact suggested by my own work is different from that suggested either by Ostwald or Richards, and is this: the physiological efficiency of the hydrogen ion is not a fixed quantity, but varies with the anion; the weaker the anion, the more powerful the action of the hydrogen. The reason for this variation, as will be discussed farther on, might possibly be that the solution tension of the positive ion is diminished and its physiological efficiency increased by a reduction in power (solution tension?) of the anion. This conclusion is not at variance with the statement of Bodländer, that the solution tension is a fixed quantity, because that statement is true only of salts completely dissociated and therefore in great dilution.

The foregoing observations, which agree with many physiological facts, establish, I believe, the relationship between solution tension, decomposition tension, and poisonous or physiological action.

The Relationship of Physiological Action to Atomic Volume.

An inspection of the table of physiological action shows that the elements do not arrange themselves according to the atomic weights. Hydrogen, for example, although the lightest of elements, is as an ion among the most poisonous. Potassium and barium are either less poisonous than sodium or have very nearly the same action. It occurred to me in considering the hypothesis that the motion of the charge determined its physiological action, that the atomic volume might give a clew to the orbit of the charge, and, assuming that the charge was moving about the atom, or the atoms themselves were rotating, with the same speed, that the atoms of large volume would

be less active than those of small volume. I therefore compared atomic volume and poisonous action. There was also a second consideration which led me to this comparison. While poisonous action is not a simple function of atomic weight, there appears to be nevertheless a certain relationship, since the general proposition that the heavier metals are more poisonous than the lighter is true. It has been shown also that atomic volume and several other functions, *i.e.*, boiling points, are *periodic* functions of the atomic weights, and hence the poisonous action might also be periodic.

TABLE VII.

METALLIC ELEMENTS ARRANGED ACCORDING TO THEIR ATOMIC VOLUME.

Element.	Atomic volume.	Element.	Atomic volume.	Element.	Atomic volume.
Cs	71	Zr	22	Ag	10
Rb	57	Te	20	Zn	9.2
K	45	Pb	18	Cu	7.2
Ba	36	Sb	18	Mn	7.3
Sr	35	Sn	16	Fe	7.2
Ca	25	Hg	15	Co	6.8
Na	23	Mg	14	Ni	6.8
Bi	21	Al	11		

Table VII shows that absolute atomic volume is not an important factor in determining poisonous action, as mercury has an atomic volume greater than magnesium. If we take the specific gravity of equivalent portions of the elements, we obtain a much closer parallelism, but one in which many exceptions occur.

A more accurate relationship between poisonous action, atomic weight, and atomic volume is the following: *The poisonous action of any metal varies directly with its equivalent weight, and inversely with its atomic volume.* This may be seen from Table VIII, although the exceptions are perhaps more numerous than in the comparison of solution tension. The elements arrange themselves in the following order:

TABLE VIII.

EQUIVALENT WEIGHTS DIVIDED BY ATOMIC VOLUMES.

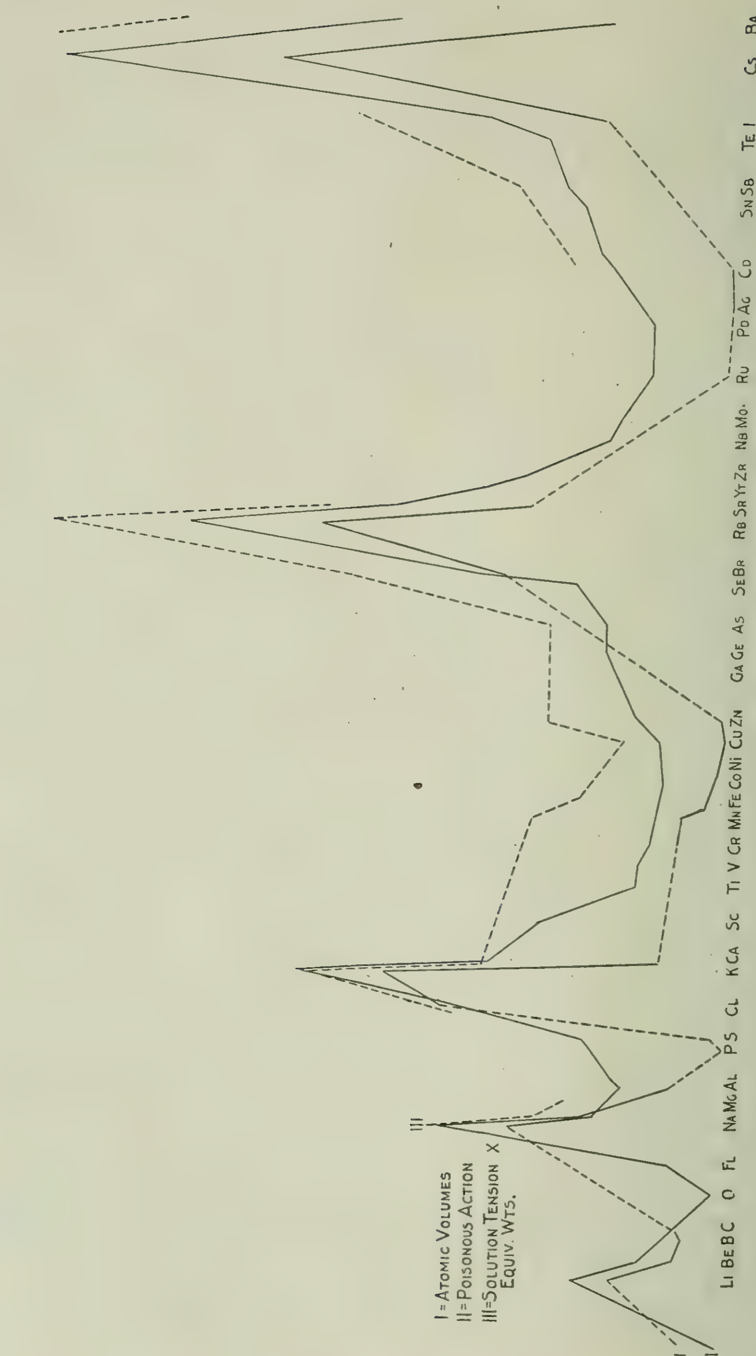
Element.	Equiv. weight. / Atomic volume.	Element.	Equiv. weight. / Atomic volume.	Element.	Equiv. weight. / Atomic volume.
Li	[illegible]	Al	[illegible]	Cd	4.3
K	[illegible]	Ba	1.8	Cu	4.4
Ca	[illegible]	Cs	1.87	Pb	5.8
Mg	[illegible]	Mn	3.8	Au	6.5
Na	1	Fe	3.9	Hg	6.3
Sr	[illegible]	Co	4.2	Ag	10.8
Rb	1.5	Ni	4.3		

This is approximately the order in which the elements occur in their poisonous action, cadmium by this relationship taking the position near copper to which its poisonous action entitles it.

The close parallelism of atomic volume and poisonous action may be seen if we plot the curves of atomic volume and weakest poisonous dose, using the atomic weights as abscissæ (Curves I and II, Fig. 1). The data for the curve of poisonous action were obtained by multiplying the concentration of the least poisonous dose (of the chlorides) by forty. The value of rubidium and cæsium are approximate, and were calculated from their solution tensions, and their action relative to potassium on nerves and other tissue.

These curves show very clearly that poisonous action, like the atomic volume, is a periodic function of the atomic weight, those elements with a high atomic volume and a low atomic weight being less poisonous than those with a low atomic volume and a high atomic weight. In Table VIII it will be seen that cadmium has, compared with its atomic weight, a very low atomic volume, and this agrees with its very powerful physiological action.

The relationship between atomic volume, solution tension, and poisonous action may also be brought out by plotting in the same manner the curve formed by multiplying the equivalent weight by the solution tension. (See Curve III.) In this way, using the value of hydrogen as the zero point, and plotting the atomic volumes of those elements which are negative, *i.e.*, chlorine, bromine, iodine,


I = Atomic Volumes
II = Poisonous Action
III = Solution Tension X Equiv. Wts.
I
II
III
Li Be B C O Fl Na Mg Al P S Cl K Ca Sc Ti V Cr Mn Fe Co Ni Cu Zn Ga Ge As Se Br Rb Sr Yt Zr Nb Mo Ru Pd Ag Cd Sn Sb Te I Cs Ba

oxygen, and so forth, as negative values, a curve is obtained in which the activity of the element increases as its ordinate, in either a positive or a negative direction, diminishes. For example, chlorine and sodium come at the extremes of the curve, and are relatively inert. The heavy metals come, as a rule, near the crossing point where the solution tension is low. By such an arrangement also, a place is found for argon and similar elements. They occur where the curve is crossing the point of zero solution tension, when travelling upward. A much more accurate comparison could be had, I believe, if we had the values for the absolute zero of solution tension.

This relationship between solution tension, atomic volume, and physiological action can be best understood, I believe, by the hypothesis recently advanced by Richards,[1] *i. e.*, that the atomic volume represents the electrical condition of the atom. This condition varies under different circumstances, and hence atomic volume and solution tension are variable quantities. To express it roughly, if the amount of electricity in the atom is low, the atomic volume is low, and its affinity for its charge (solution tension) is also low, so that its chemical and physiological affinity is high. This suggestion of Richards, in support of which he has brought forward many facts, is of great importance to the pharmacologist and the physiological chemist. If it is true, it indicates *that the physiological efficiency of any ion is not a fixed quantity, except in infinite dilution.* At the present time, we assume that potassium and chlorine as ions have given actions, no matter what may be the ions with which they are associated. A similar assumption underlies the work on solution tension, and this is in entire harmony with the current hypothesis, that the ions move independently of each other. While this may be true for ions in exceedingly dilute solutions, it may not be true for more concentrated solutions. Is the affinity of chlorine for its charge in equivalent ionic solutions of chlorides always the same, regardless of whether chlorine is combined with a metal like potassium or with mercury; with an ion with a high solution tension or with a low one? If the foregoing suggestions concerning the relationship between solution tension and atomic volume are true, it appears to me that in anything but very dilute solutions the atomic volume of any negative ion may increase when brought near any strongly positive ion like potassium, and may decrease when brought

[1] RICHARDS: Zeitschrift für physikalische Chemie, 1902, xl, p. 172.

near a weak ion like hydrogen or mercury, as is indicated in Richards's result for the solids; and also that the affinity of chlorine for its charge may be greater, and hence its physiological activity be less, when in combination with potassium than when it is in combination with sodium, or calcium, or mercury.

If such an action of one ion upon another as is indicated by the above reasoning really exists, it offers an explanation of the undoubted fact that the physiological action of hydroxyl, for example, or any other ion, varies with the ion with which it is combined. This fact is capable of being explained on the basis of the independence of the ions as has already been stated (see pages 309, 310). We may assume that potassium, for example, or any other positive ion, acts on the protoplasm in such a way as to make it less sensitive to the action of the anion. The fact is, that the higher the solution tension of the positive ion, the more completely does it neutralize the action of the opposite ion. This explanation, however, is unsatisfactory, for the reason that it does not indicate *how* the ion can act to produce this insusceptibility to the action of the anion. Were the action on the protoplasm, one would expect, inasmuch as the physiological action of an anion is inversely proportional to the solution tension, that hydrobromic and hydriodic acid would be less poisonous than hydrochloric, since their anions, having a lower solution tension, should more successfully counteract the action of the hydrogen. As a matter of fact, these acids are more poisonous. The antitoxic action of opposite ions being apparently directly proportional to the solution tension, indicates that the ions act on each other. In my opinion the facts here cited regarding the modifying action of the two ions are best understood on the hypothesis that the two ions act on each other and on protoplasm in the same manner. Potassium hydrate is less poisonous than sodium hydrate, because in the presence of the powerfully positive element potassium, the amount of electricity, if one may so express it, in the hydroxyl is increased, its ionic (atomic) volume increases, and it holds its negative charge more firmly. Its physiological efficiency is thereby diminished. Similarly, in the presence of potassium, the protoplasmic molecules may possibly be made to hold their charges more firmly, and as I have already shown, their irritability is thereby reduced,

The fact that the physiological action of the elements may be calculated with approximate correctness from their solution tension, and also, although less certainly, from a ratio between atomic volume

and equivalent weight, indicates that both atomic volume and atomic weight factors are already represented in the solution tension figures.

Conclusions and Summary.

The establishment of the general conclusions that the affinity of the atom or ion for its charge is one of the main factors which determines its physiological action, suggests that inorganic salts may act on protoplasm in the following way:

Adopting the one fluid hypothesis of electricity, a positive ion may be regarded as one having an unsaturated affinity for a negative charge or electron. We may conveniently assume that sodium and chlorine are held together, in part at least, by the affinity each has for a negative charge or electron. When the salt goes into solution, the ions separate, and chlorine, having the greater affinity for the negative charge, steals it away, leaving the sodium or potassium a charge short. The chlorine, in other words, reduces the sodium, or, as more generally stated, oxidizes it. The different positive ions have very unequal affinities for their charges, some of them, like potassium, holding the positive charge very strongly, others, like mercury, holding the positive charge very weakly, and having a corresponding great affinity for a negative charge. The positive ion, with its unsaturated affinity, comes in contact with a protoplasmic molecule or colloidal particle which contains at some point a negative charge. If it has a greater affinity for the charge than the protoplasm has, it will steal it away, and become at once an atom of sodium.

When the protoplasmic particle loses this charge, it changes its chemical or physical shape. If the charge comes from the surface of a colloidal particle, a reduction of surface will take place, leading thereby to movements within the protoplasm, due to a change in the state of aggregation.[1] If the charge is taken from a protoplasmic molecule or a complex ion, chemical decompositions will ensue, just as happens when proteid or any other complex organic substance is broken up in electrolysis. By this reduction of the protoplasm, chemical decompositions, analogous in all respects to electrolytic decompositions, are brought about, energy is set free, and movements

[1] This explanation necessarily implies that the results hitherto obtained by Hardy and others, on colloidal solutions in which coagulation is set forth as a function of valence, will have to be reinterpreted from this solution tension point of view.

of the protoplasm result. The protoplasmic decompositions and syntheses are, hence, on this hypothesis, probably electrolytic in nature, and the comparisons long ago made by Drechsel and Baumann, between the action of rapidly alternating currents on solutions of albumin, urea, and other substances, and the decompositions and syntheses brought about by protoplasm, is a correct comparison; the processes are identical in nature. The electrically charged ions play the part of minute electrodes of different voltages in the protoplasm.

It is clear that the activity of the positive ion will increase with an increase in its affinity for a negative charge or a diminution in its solution tension, — a relationship borne out by the facts presented in this paper, and other facts well known to all biologists. Hence it happens that an ion like copper, mercury, or silver, which has a powerful affinity for negative charges, or which gives up the positive charge easily, is able to bring about far more deep-seated changes, more fatal for protoplasm, than an ion like potassium, which has a very weak affinity for such negative charges. Sodium or potassium are able to steal away only those charges which are held least firmly.

Furthermore, when sodium or any other positive ion loses its positive charge, and acquires a negative charge, it becomes an atom, and, acting on the water, will give up its charge to hydrogen, which thereby becomes nascent hydrogen with an intense chemical action. The sodium ion is regenerated, having taken the positive charge from the hydrogen, and an hydroxyl ion is formed. We thereby have a basis for the oxidative and respiratory functions of protoplasm. In the course of the decompositions thus brought about, carbon dioxide will probably be formed, just as it is formed in the electrolysis of propionic acid and similar substances. Some of these substances may have a greater affinity for the negative charge of the sodium atom than sodium, and thus assist in regenerating the ion. In this way it is possible to see how a very small amount of an ion can bring about an enormous physiological change, such as the rhythmic contraction of muscle. It is, in fact, a ferment action. The weaker the ion is in its affinity for a positive or negative charge, the greater should be its catalytic action.

While I have thus far considered only the positive ions, it is clear that the negative ions are acting and must act in exactly the same manner, but to an opposite result. We have seen that those negative

ions are most powerful which part with their negative charges most readily. Such ions as oxygen or iodine will give up their charges with great ease to protoplasm. The greater their affinity for positive charges, the easier do they part with the negative. While, therefore, the positive ions reduce protoplasm (oxidize it in the ordinary sense), and act like ionic hydrogen, the negative ions will oxidize protoplasm like hydroxyl ions, giving up to it negative charges (oxidation being an increase in negative charges).[1]

It has long been known that an increase in the alkalinity of protoplasm favored oxidation, while a decrease caused respiration to cease. The foregoing facts and reasoning indicate an explanation for this action. Respiration is carried on chiefly by the oxygen ions. When these are increased in number, as they are increased by making protoplasm more alkaline, their solution tension falls; they give up their negative charges so much the more rapidly and easily. When, on the other hand, we increase the acidity, or reduce the alkalinity, there is a reduction in hydroxyl ions, their solution tension thereby increases, they no longer give up negative charges to protoplasm, and respiration is brought to a stop. This concerns chiefly the hydroxyl and oxygen ions, because these are among the ions present in protoplasm with the lowest solution tension. Anærobic life does not differ hence from ærobic. Life and respiration of any cell is checked as soon as the number of hydroxyl ions is reduced so far that the solution tension of the ion is greater than the solution tension of the protoplasm.

Finally, it is clear that the physiological action of the metal or of the element, when it has a positive charge, must be the reverse of the action of the same element when it has a negative charge. Ionic chlorine, for example, will have an opposite physiological action from atomic chlorine, and this may readily be observed. Atomic chlorine acts like a powerful positive ion, quickly anæsthetizing nerves. It is in most circumstances a powerful depressant, though for some kinds of protoplasm it is an excitant. Ionic chlorine has an opposite action. Similarly atomic and ionic iodine have opposite actions. Potassium iodide has the depressant action of the potassium and atomic iodine. The iodine ion loses its charge in the body with much greater ease than chlorine, so that when the iodides are given, we are dealing not

[1] I have used the term oxidation here as equivalent to an increase in negative charges because it seems to me this use of the term is preferable to its ordinary meaning of an increase in positive charges.

alone with the action of the ion, but with the atom also. This is true to a less degree of bromine, and to a still less degree of chlorine, though conditions may and do arise in the body which result in the reduction of the chlorine and its retention in the non-ionic form, as, for example, in pneumonia.

These considerations and conclusions throw, I think, a new light on the relation of inorganic salts to protoplasmic activity, and show how fundamentally important they are. While life may, in some instances, go on in their almost entire absence, the ions in water being sufficient for some forms of protoplasm, yet the salts present furnish the electrical substratum in which the phenomena of life are played. From them electrical charges can be obtained, and to them given up.

One other conclusion may be drawn from the work which is incorporated in this paper, and from that on nerve stimulation, which, I hope, will soon be published. The striking fact appears from the study of the action of organic and inorganic compounds on protoplasm and protoplasmic fermentations, that chemical composition, as such, is of little or no importance in determining physiological action. This comes out, perhaps, most clearly in a study of compounds which taste sweet. Such widely different substances as beryllium sulphate, lead acetate, sugar, phloroglucin, and saccharine taste sweet. This fact teaches us how hopeless it is to attempt to explain physiological action by molecular structure or chemical composition. We have to seek that something which lead acetate and sugar and saccharine have in common, and this we may most readily do by studying the simple inorganic salts. From studies of which this paper is a part, it appears that the physiological action of lead or any other inorganic salt is determined by the character and number of its electrical charges and by its solution tension, or ease of parting with those charges. Lead acetate possesses, therefore, a certain electrical condition, and it is that which gives it its properties. We may confidently assume that the sugar molecule must possess in certain respects a similar electrical state, since it acts in the same manner. In my opinion the fact that such widely different bodies taste sweet, coupled with the facts already stated, shows the futility of attempting to explain the relation of a ferment to a fermentable substance, or of a toxine to its anti-body on a structural chemical basis. The lock and key conception, and Ehrlich's hypothesis must, hence, be entirely relinquished. What determines the action of a ferment, or a toxine, if my conclusions

here outlined are correct, is neither chemical composition nor chemical structure, but a certain electrical or physical condition of the molecule or atom, a condition which may be the same or closely similar, in substances which, chemically, are totally different.

Summary.

1. The physiological action (poisonous action) of any cation or metal upon Fundulus eggs and probably other forms of protoplasm varies inversely with the solution tension. Those ions with a very low solution tension are very poisonous; those with a high tension are relatively inert.

2. The poisonous action of any anion is similarly an inverse function of the solution tension; oxygen, cyanogen, oxalate, and iodine ions, with a low solution tension, being more poisonous than chlorine with a high solution tension.

3. The poisonous action (physiological action) of any salt is, therefore, a function of both ions, and varies inversely with the sum of the solution tensions of the ions, *i. e.*, with the decomposition tension of the salt.

4. From the decomposition tension of two salts, knowing the minimum fatal dose of one, the minimum fatal dose of any other may be calculated approximately, in many instances, at any rate, by the following formula:

$$V_a = \frac{V_o}{2^{\frac{E_a - E_o}{0.14 + 0.03\,E_a}}}$$

in which V_a is the dilution of the unknown minimum fatal dose of some salt, E_a is the decomposition tension of this salt; V_o is the known dilution of the minimum fatal dose of some salt; and E_o, the decomposition tension of this salt.

5. The exceptions to the foregoing statement are, in the negative ions, primarily fluorine, which is credited with a solution tension for its negative charge higher than chlorine, and the oxygen ion, and among the metals, cadmium. Cadmium[*] is nearly as poisonous as copper. This indicates either that the cadmium was impure, or the solution tension of one of its charges is much lower than that of the other. No experimental evidence of this could be found, however, beyond the tendency to form double salts and its low dissociation. Other exceptions were noted.

6. There is a marked difference between ferrous and ferric salts. This is due to the fact that the solution tension of ferric salts is very low, being near that of copper. This is shown, first, by the low heat of formation of ferric chloride, and, second, by the fact that in the ferric state the ferric ion has a greater affinity for a negative charge than has iodine, and is thus able to throw free iodine from a solution of its salts. The ferrous ion is not able to do this. The quantitative divergence of the results obtained for nickel, cobalt, and ferrous ions, from those computed from the solution tension, are probably to be ascribed to the difficulty of penetration into the egg, or to unknown variations in the quantity and composition of the ions present in such strong solutions.

7. The physiological action of any ion or atom (?) is, hence, determined by its solution tension, or its affinity for its charge. Mercury, silver, and copper are poisonous, because they part with their charges to the protoplasmic particles easily, thereby bringing about changes in the state of aggregation of the colloidal particles, and decomposition of the molecules, — in other words, physical and chemical changes leading to movements, and so on. The changes thus produced are electrolytic in nature, and we thus have strong evidence that the salts act by their electrical charges, and that the protoplasmic decompositions and syntheses are electrolytic in character.

8. There is an inverse relationship between atomic volume and poisonous action; and a direct relationship between equivalent weights and poisonous action. Poisonous action of the metals is, hence, a periodic function of the atomic weights, just as is atomic volume. Elements with a low atomic volume and high equivalent weight (mercury) are greatly more active than those with a high atomic volume and a low equivalent weight (sodium).

9. As there is reason for believing that atomic volume varies (Richards), the physiological efficiency of any ion is, hence, not a fixed quantity, but probably varies in strong solutions with every ion of an opposite character with which it is associated. This gives a possible explanation of the fact that a marked difference exists in the poisonous action of different hydrates containing the same number of hydroxyl ions, and of acids containing the same number of hydrogen ions.

10. The results obtained indicate that the solution tension of any ion becomes greater, and its physiological action consequently less, the higher the solution tension of the opposite ion with which it is

associated. Thus all potassium salts were found less poisonous than the corresponding sodium salts.

11. The poisonous action of potassium for special tissues, *i.e.*, muscle and nerve, is possibly due to special conditions and must be further investigated.

ON THE PRODUCTION OF CONTACT IRRITABILITY WITHOUT THE PRECIPITATION OF CALCIUM SALTS.

By W. D. ZOETHOUT.

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IN a former paper[1] on the contact irritability of muscles, it was shown that potassium chloride increases the contact reaction brought about by sodium salts whose anions precipitate calcium, such as the fluoride, oxalate, tartrate, and citrate. Previous to that it was found that potassium salts increase the tonicity of skeletal muscles and that calcium chloride and sodium chloride antagonize this action of the potassium salts.[2] Subsequent work[3] showed that what is true for potassium chloride is to a greater or less extent also true for the chlorides of ammonium, cæsium, and rubidium. All these salts increase the tone of the gastrocnemius muscle of the frog, and the tone thus developed is reduced by the subsequent application of calcium, strontium, and magnesium chloride, and to some extent by sodium and lithium chloride. This similarity of action led me to determine the influence of these salts on the production of contact irritability. Here also the similarity in physiological action of potassium, cæsium, rubidium, and ammonium salts and of sodium and lithium salts appears very strikingly, as the following experiments show.

In these experiments the gastrocnemius muscle of the frog was used. In case it was necessary to compare the action of the two salts, the two gastrocnemius muscles of one and the same frog were used. The muscles were prepared with the utmost care in order to avoid all injury. All solutions used were $\frac{m}{8}$ solutions, unless otherwise stated.

Ammonium chloride. — The first salt whose influence on contact reaction was tested was ammonium chloride. One of the gastrocne-

[1] ZOETHOUT: This journal, 1902, vii, p. 320.
[2] ZOETHOUT: This journal, 1902, vii, p. 199.
[3] ZOETHOUT: This journal, 1904, x, p. 211.

mius muscles was placed in 10 c.c. $\frac{m}{8}$ ammonium chloride, while the other was placed in 10 c.c. $\frac{m}{8}$ sodium chloride. After two or three minutes both muscles were immersed in a solution of 8 c.c. $\frac{m}{8}$ NaFl + 2 c.c. $\frac{m}{8}$ NaCl. As I wished to determine the minimum concentration of sodium fluoride which was still able to call forth contact reaction in these two muscles, it was necessary to use solutions which do not produce this reaction very rapidly in the normal muscle; hence the sodium fluoride was diluted with sodium chloride solution. On exposure to air, the muscle previously treated with ammonium chloride showed a slightly better contact reaction than the muscle which had been treated with sodium chloride. The action of ammonium chloride was also compared with that of moist air. In this case the control muscle was placed in the narrow glass tube which formed a part of the apparatus. This tube was closed at the bottom by means of a cork and a thin layer of 0.7 per cent sodium chloride solution was placed in it, while the top was closed by moist filter paper. In this way the air in the tube was kept moist. Here also the muscle treated with ammonium chloride solution for two or three minutes produced a better contact reaction than the control muscle. This favorable action of ammonium chloride was further demonstrated by comparing the effect of 2 c.c. $\frac{m}{8}$ NH_4Cl + 8 c.c. $\frac{m}{8}$ NaFl with that of 2 c.c. $\frac{m}{8}$ NaCl + 8 c.c. $\frac{m}{8}$ NaFl.

Sodium chloride. — After a few of the above experiments it was observed that sodium chloride solution is not indifferent towards contact irritability. This led me to test the action of this salt as compared with that of moist air, and I found that a muscle treated for five or six minutes with $\frac{m}{8}$ sodium chloride has a less powerful contact reaction than one remaining for an equal time in moist air. This inhibiting action of sodium chloride was also shown by comparing the effect of 2 c.c. $\frac{m}{8}$ NaCl + 8 c.c. $\frac{m}{8}$ NaFl with that of 2 c.c. H_2O + 8 c.c. $\frac{m}{8}$ NaFl.

Lithium chloride. — Lithium chloride also has a tendency to decrease the contact reaction as compared with the effect of moist air. The inhibiting power of lithium chloride is slightly greater than that of sodium chloride.

Magnesium chloride. — As we would expect from its action on skeletal muscles, this salt has a great inhibiting influence on contact irritability. A muscle was placed for six minutes in $\frac{m}{8}$ magnesium chloride solution while the control muscle was left in moist air for the same length of time. At the expiration of this time, both muscles

were placed in a bath of 2 c.c. $\frac{m}{8}$ NaCl + 8 c.c. $\frac{m}{8}$ NaFl. The control muscle showed powerful contact reaction in three or four minutes while the muscle treated with magnesium chloride did not show a trace of contact reaction even after remaining for twenty minutes in the sodium fluoride solution.

Barium chloride. — From its chemical relationship to calcium chloride we would expect this salt to have an inhibiting influence on contact reaction. And, no doubt, strong solutions of this salt or weaker solutions acting for a long time do destroy contact irritability; but on comparing the action of very dilute solutions of barium chloride with that of sodium chloride solution, it was found that the contact reaction brought about by the subsequent application of 2 c.c. $\frac{m}{8}$ NaCl + 8 c.c. $\frac{m}{8}$ NaFl is better in the muscle treated with the dilute barium chloride than in the muscle treated with sodium chloride. The degree of dilution used was $\frac{1}{2}$ c.c. $\frac{m}{8}$ $BaCl_2$ + $9\frac{1}{2}$ c.c. NaCl, or, better still, 0.1 c.c. $BaCl_2$ + 9.9 c.c. NaCl; these solutions were allowed to act for two or three minutes.

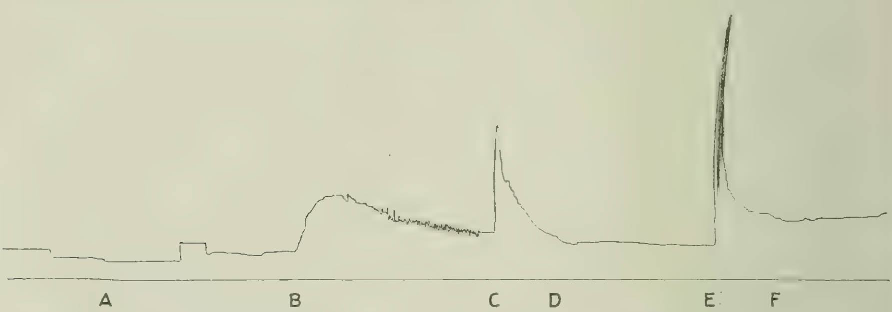

FIGURE 1. — At A, muscle was placed in 2 c.c. $\frac{m}{8}$ RbCl + 8 c.c. $\frac{m}{8}$ NaCl. At B this was exchanged for 2 c.c. NaCl + 8 c.c. $\frac{m}{8}$ NaFl. At C and E this fluid was removed, and at D and F replaced.

Rubidium and cæsium chloride. — Of all the salts which aid the production of contact irritability, rubidium and cæsium chloride are the most active. The action of these salts was compared with that of sodium chloride solution, water, and moist air, and in all three cases it was found that the muscle treated with rubidium or cæsium chloride excelled in contact reaction those not thus treated. This is well illustrated in Figs. 1 and 2. In Fig. 1 one of the gastrocnemius muscles of a frog was placed at A in a solution of 2 c.c. $\frac{m}{8}$ RbCl + 8 c.c. $\frac{m}{8}$ NaCl, while in Fig. 2 the other gastrocnemius was placed at A in 10 c.c. sodium chloride. At B both solutions were exchanged for 2 c.c.

NaCl + 8 c.c. $\frac{m}{8}$ NaFl. At C and E, in both figures, the solutions were removed, thereby exposing the muscle to the air. In Fig. 1 the muscle showed good contact reaction; the muscle in Fig. 2 hardly showed a trace of it. The same results were obtained when the control muscle was placed in moist air.

Instead of sodium fluoride as in the above experiments, sodium sulphate was also used in conjunction with rubidium chloride. As Loeb[1] states, this salt does not always cause contact reaction in the normal muscle, although it is a sodium salt which, partly at least,

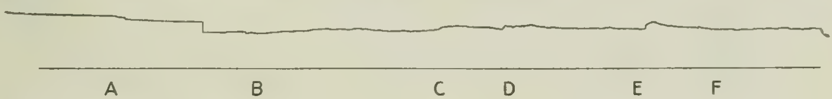

FIGURE 2. — At A the muscle was placed in $\frac{m}{8}$ NaCl. At B this was exchanged for 2 c.c. $\frac{m}{8}$ NaCl + 8 c.c. $\frac{m}{8}$ NaFl. At C and E this fluid was removed, and at D and F replaced.

precipitates the calcium. When, however, the muscle was previously treated with rubidium chloride, sodium sulphate never failed to bring about a speedy and powerful contact reaction.

This extremely favorable action of rubidium and cæsium chloride led me to surmise that perhaps by means of these salts I might be able to cause contact reaction in a muscle by using in conjunction with them some salt which precipitates calcium but whose cation is not sodium. Loeb had found that "with one exception only sodium salts give rise to contact irritability, and this exception is a sulphate, namely $(NH_4)_2SO_4$."[2] To prove this, we began with the sulphate of lithium. After determining the most favorable concentration of both the lithium sulphate and the rubidium chloride, it was found that a solution of 2 c.c. $\frac{m}{8}$ RbCl + 8 c.c. $\frac{m}{8}$ Li_2SO_4 also produced contact reaction. The same was found true, although to a lesser extent, of lithium citrate. Next the ammonium salts were tested. As was said above, Loeb found that the sulphate caused contact irritability, the carbonate and citrate he found to be inactive. I found, however, that a solution of 0.4 c.c. $\frac{m}{8}$ RbCl + 9.6 c.c. $\frac{m}{8}$ ammonium oxalate gave rise to some contact irritability. This reaction disappears very speedily. Ammonium citrate also gave rise to a feeble contact reaction.

In the experiments thus far related, all the salts used to bring

[1] LOEB: This journal, 1901, v, p. 362. [2] LOEB: *Ibid.*

about contact irritability possess anions which form insoluble calcium compounds, such as the sulphates, oxalates, fluorides, etc. In fact, Loeb states that "the salts whose solutions produce this form of irritability [contact irritability] are (with one exception) sodium salts, whose anions are liable to precipitate calcium." To ascertain whether, even with the use of rubidium chloride, the salts must have the power to precipitate calcium, I used the acetate, succinate, and nitrate of sodium, salts which Loeb found were unable by themselves to cause this reaction. I found that if these salts are mixed with rubidium or cæsium chloride, they give rise to powerful contact reaction. The most active solution was found to be 0.4 c.c. $\frac{m}{8}$ RbCl + 9.6 c.c. of the

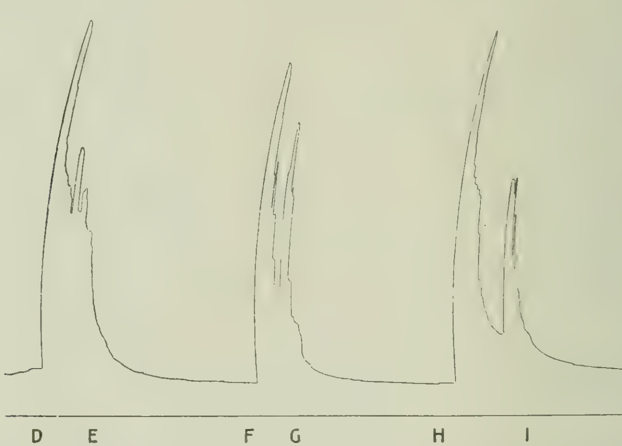

FIGURE 3.— Contact irritability produced by $\frac{4}{10}$ c.c. $\frac{m}{8}$ RbCl + $9\frac{6}{10}$ c.c. $\frac{m}{8}$ sodium acetate. At D, F, and H the solution is removed. At E, G, and I it is replaced.

sodium salt (see Fig. 3). Of the three above-named salts, sodium acetate gave the best and the nitrate the least action.

Concerning the chloride, iodide, and bromide of sodium, it may be stated that sodium chloride never produced any contact reaction, no matter what the proportion of rubidium chloride and sodium chloride used; a solution of 1 c.c. $\frac{m}{8}$ RbCl + 9 c.c. $\frac{m}{8}$ NaI in some cases gave a feeble contact reaction, not to be compared, however, with that produced by the acetate or succinate of sodium; sodium bromide had practically no effect.

The action of sodium phosphate was next tried. This salt, of course, precipitates calcium, but concerning its action Loeb[1] says: "If we

[1] LOEB: *Loc. cit.*

put the muscle into a solution of sodium phosphate, it goes at once into a powerful tetanus. . . . When a muscle goes into tetanus *in* a solution, we cannot, as a rule, demonstrate contact reaction. Thus I have never succeeded in producing contact reaction by a sodium phosphate solution." By using a solution of 0.4 c.c. $\frac{m}{8}$ RbCl + 4.6 c.c. $\frac{m}{8}$ Na_3PO_4 + 5 c.c. H_2O the increase of tone of the muscle in the solution itself is very small, and the subsequent contact reaction, when the muscle is removed from the solution, is exceedingly powerful. In fact, such a perfect contact reaction is induced by this solution, that I was led to test the action of sodium phosphate itself. In order not to throw the muscle into tetanus, thereby preventing contact reaction, a $\frac{m}{16}$ solution of sodium phosphate was used and a very powerful contact reaction was obtained. When the $\frac{m}{8}$ sodium phosphate is diluted with sodium chloride, the contact reaction is far less powerful; this is in harmony with what was previously said in regard to the inhibiting influence of sodium chloride.

Having shown that certain salts of sodium which do not precipitate calcium may cause contact irritability in a muscle treated with rubidium or cæsium chloride, it was then necessary to discover whether the sodium ions are indispensable for this reaction. As lithium chloride is very similar to sodium chloride in its action on contact irritability, and as lithium citrate and sulphate (which precipitate calcium) cause contact irritability when they are used in conjunction with rubidium or cæsium chloride, the action of certain lithium salts which do not precipitate calcium was investigated. Lithium acetate and nitrate were used. The acetate gave a slight amount of contact reaction when used with rubidium chloride; the nitrate had no effect at all. Neither did lithium iodide have any action. The lithium salts, therefore, are not nearly so efficacious in the production of contact irritability as the sodium salts, and this is in harmony with what was said before concerning the relative strength of the antagonistic action of sodium and lithium chloride toward contact reaction. The action of ammonium acetate and nitrate were next tested, but neither salt had any power to generate contact irritability even with the aid of rubidium or cæsium chloride. Magnesium tartrate and sulphate, although precipitating calcium, gave no better results. When it is borne in mind what was said of the great inhibiting action of magnesium chloride upon contact reaction, we would hardly expect the tartrate and sulphate to cause this reaction even with the help of rubidium chloride.

Seeing that rubidium and cæsium chloride act thus favorably in the establishment of contact irritability when used in conjunction with certain other salts, it was naturally suspected that these chlorides themselves might be able to produce this irritability. Muscles were, therefore, subjected to solutions of rubidium and cæsium chloride varying in strength from $\frac{m}{8}$ to $\frac{m}{130}$, the salts in some cases being diluted with water, in others, with $\frac{m}{8}$ urea solutions, but in all cases the results were negative.

Potassium chloride. — Having obtained such favorable results with rubidium and cæsium chloride, attention was turned to potassium chloride, which, as I have shown before,[1] increases the contact reaction brought about by sodium salts which precipitate calcium. On comparing the effect of this salt with that of either rubidium or cæsium chloride, it was found to be far inferior in aiding the genesis of contact irritability. The contact reaction brought about by potassium chloride mixed with sodium acetate or succinate or with lithium sulphate was very slight compared with that produced by rubidium chloride when mixed with these salts.

General Considerations.

From the foregoing it is clear that the salts which induce contact irritability can be grouped in such a manner as to show their relative strength.

TABLE I.

Class I	Group I	Sodium salts whose anions precipitate calcium. Ammonium sulphate.
	Group II	Sodium acetate. Sodium succinate. Sodium nitrate.
	Group III	Lithium sulphate. Lithium citrate. Ammonium oxalate. Ammonium citrate.
	Group IV	Lithium acetate.
Class II		Bromide, iodide, and chloride of sodium. Iodide and nitrate of lithium. Nitrate and acetate of ammonium. Sulphate and tartrate of magnesium.

[1] Zoethout: This journal, 1902, vii, p. 320.

In this table the salts are arranged approximately in the descending order of their power to cause contact reaction.

The salts in Group I can produce contact irritability without the intervention of any other agency. In Group II are found the sodium salts whose anions do not precipitate calcium. While these salts by themselves do not produce contact irritability, with the aid of cæsium or rubidium chloride they readily do so. The element common to this and the preceding group is the sodium ion. In Group III are found the salts of other metals than sodium, namely, lithium and ammonium, whose anions precipitate calcium and which cause contact irritability with the aid of cæsium or rubidium chloride. While these salts differ from those of Group I in not containing sodium, they resemble them in that they precipitate calcium. In Group IV are the salts of lithium which do not precipitate calcium and yet with the aid of cæsium or rubidium chloride produce contact irritability. All these salts, grouped together as Class I, give rise to contact reaction, either with or without the intervention of some other salt.

In Class II are included the salts that do not cause contact reaction even with the aid of cæsium or rubidium chloride. In regard to the sodium iodide and bromide it may be repeated that occasionally they give rise to a very slight amount of contact reaction when the muscle is simultaneously treated with rubidium chloride. They may, therefore, be regarded as transitional forms between Class I and II.

The salts that aid the genesis of contact irritability brought about by the salts mentioned in Class I of the preceding table may also be tabulated. They may be arranged in the order of their lessening power as follows:

TABLE II.

Class A.	Class B.
Rubidium chloride.	Sodium chloride.
Cæsium chloride.	Lithium chloride.
Potassium chloride.	Magnesium chloride.
Ammonium chloride.	Calcium chloride.
Barium chloride.	Strontium chloride.

The salts in Class B delay and even prevent the onset of contact irritability; those in Class A aid in the production of this irritability. The power of barium chloride is extremely limited, and that of ammonium chloride is not very marked.

From this it is evident that, under the proper circumstances, sodium, lithium, and ammonium salts can give rise to contact irrita-

bility. Of these the sodium salts are the most active and the ammonium salts the least. Of the sodium salts, those that precipitate calcium are the most active; in fact, it is only these salts (with the exception of ammonium sulphate) that can give rise to contact irritability without the intervention of any other agent. Of those salts that do not precipitate calcium, only the sodium and lithium are active, and then only when a member of Class A is present. Ammonium salts can cause contact irritability only if they precipitate calcium and if a member of Class A is present (except the sulphate).

The precipitation of calcium, then, seems to be one of the conditions by which contact reaction may be produced. To this Loeb draws attention when he says, "that a decrease in the amount of calcium-ions in the tissues (and possibly an increase in the amount of sodium-ions) is the essential condition for the production of contact reaction."[1] This influence exerted by the decrease of calcium ions can be best explained, it seems to me, by supposing that the calcium normally found in the muscle acts as an inhibiting agent upon the activity of some other agent or agents. In a former paper I pointed out how this agent may possibly be the potassium salt.[2] This view is strengthened and broadened by the above experiments in proving that not only potassium, but also ammonium, cæsium, and rubidium have the power to increase contact reaction. These substances — potassium, ammonium, cæsium, and rubidium — which have been classed together in Class A are substances that increase the tonicity of skeletal muscle, as shown in a recent paper.[3]

Loeb found that "by adding a small amount of $CaCl_2$ to a Na-citrate solution the latter solution no longer produces the contact reaction."[4] I found that not only calcium chloride but also the chlorides of strontium and magnesium, and to a lesser extent sodium and lithium chloride have this effect. I have also shown that these substances decrease the tone of skeletal muscles and abolish the increase in tonicity caused by potassium, cæsium, etc. It seems, therefore, that those substances that increase the tone aid in the production of contact irritability, and that those substances that antagonize the increase of tone also antagonize the genesis of contact irritability.

[1] Loeb: *Ibid.*
[2] Zoethout: This journal, 1902, vii, p. 320.
[3] Zoethout: This journal, 1904, x, p. 211.
[4] Loeb: This journal, 1901, v, p. 366.

Salts of Group II, III, and IV must be accompanied by some salt of Class A (preferably cæsium or rubidium chloride) in order to establish contact reaction. The salts of Group I do not need to be accompanied by any salt of Class A, because the normal muscle itself contains sufficient salts of this nature, provided they are allowed to act, as they are by the precipitation of calcium brought about by the very salt used. The question then naturally arises why, in order to establish contact reaction, must salts of Class A be added to salts of Group III (lithium sulphate, etc., and not to those of Group I (sodium sulphate, etc.), seeing that both these groups precipitate calcium. This, I think, can be answered by assuming that in addition to a substance which increases the tonicity, a second substance must be present in order to produce contact reaction. When salts of Group II are used (in addition to the cæsium or rubidium chloride) this second substance is the sodium salt; when salts of Group IV are used it is the lithium salt. But it has already been stated that the salts of lithium which do not precipitate calcium do not cause as powerful contact reaction as the corresponding sodium salts. The lithium salts are, therefore, less able to act the part of this second agent than the sodium salts; this is in harmony with the fact that chloride of lithium inhibits the contact reaction produced by sodium fluoride more than the chloride of sodium. It is for this reason that the lithium salts that precipitate calcium cannot by themselves produce contact reaction, although the corresponding sodium salts can. Moreover, it has been shown that lithium chloride antagonizes the action of potassium, cæsium, etc., more than sodium chloride.[1] Hence, in order that a lithium salt shall cause contact irritability, it is not sufficient that the calcium in the muscle be precipitated, so that the potassium and other substances in the muscle can be active, but this must be reinforced by the introduction of some substance such as cæsium or rubidium chloride.

The ammonium salts are still less active, and only such salts as precipitate calcium can give rise to contact irritability, and then only in the presence of a salt of Class A. The position of the ammonium salts is, in fact, peculiar in that they are the only salts found in both Class I and A. They slightly increase the contact reaction produced by such salts as sodium fluoride, oxalate, etc., and in conjunction with rubidium or cæsium chloride they (the oxalate

[1] ZOETHOUT: This journal, 1904, x, p. 211.

and citrate, for example) can give rise to contact irritability. But in both cases, the action of these salts is slight. They are, therefore, transitional forms between Class I of Table I and Class A of Table II.

Whether the activity of the second agent (*e.g.*, sodium nitrate or lithium acetate) is due to the anion or the cation, or to both, I cannot at this stage determine. Further experiments are being made to decide this.

Summary.

1. The chlorides of potassium, cæsium, ammonium, rubidium, and perhaps barium aid the development of contact irritability, although these salts themselves do not give rise to this form of irritability. The chlorides of sodium, lithium, magnesium, and calcium inhibit the genesis of contact irritability.

2. In the normal muscle, only those salts of sodium that precipitate calcium can produce contact irritability. But if cæsium or rubidium chloride are introduced into the muscle simultaneously with the acetate, succinate, or nitrate of sodium, contact irritability is established.

3. This also holds true for the oxalate, citrate, and sulphate of lithium and ammonium and to a slight extent for lithium acetate.

4. The following salts do not cause contact irritability even with the aid of cæsium or rubidium chloride: the bromide, iodide, and chloride of sodium and lithium, the nitrate of lithium and ammonium, the acetate of ammonium, and the sulphate and tartrate of magnesium.

EFFECT OF IONS ON THE DECOMPOSITION OF HYDROGEN PEROXIDE, AND THE HYDROLYSIS OF BUTYRIC ETHER BY A WATERY EXTRACT OF PANCREAS.

By C. HUGH NEILSON and ORVILLE H. BROWN.

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IT has been many times observed that the action of individual enzymes may be either accelerated, diminished, or totally abolished by the use of salt solutions. Kubel[1] found that sodium chloride at certain concentrations accelerates the action of ptyalin in splitting starch paste into sugar. Oppenheimer[2] says, in general, dilute salt solutions accelerate fermentative processes; but increased concentrations inhibit, and when the solutions are sufficiently concentrated the action is stopped. Dumas[3] claims that borax inhibits the action of all enzymes. Sodium chloride and ammonium sulphate in strong concentrations, according to Grützner,[4] inhibit peptic digestion. It was found by Podolinski[5] that all salts accelerate the action of trypsin, but that the effect varies, the sodium salts being the most active. According to Oppenheimer,[6] most neutral salts and a few of the heavy metals delay the action of emulsion. Lintner[7] says that the salts of the alkalies and alkaline earths hinder the action of diastase. This idea is opposed to that of A. Mayer and others who assert that sodium chloride accelerates the action of diastase, but that calcium chloride inhibits the action of the same enzyme. Kjeldahl[8] found

[1] KUBEL: Archiv für die gesammte Physiologie, 1899, lxxvi, pp. 276–305.

[2] OPPENHEIMER: Die Fermente und ihre Wirkungen," 1900, p. 40.

[3] DUMAS: Comptes rendus, 1872, lxxv, p. 295 (reference from OPPENHEIMER'S "Die Fermente und ihre Wirkungen," p. 40).

[4] GRÜTZNER: Archiv für die gesammte Physiologie, 1876, xii, pp. 285–305.

[5] PODOLINSKI: Beitrag zur Kenntniss der Pancreas-Eiweissverdauung, Dissertation, Breslau (from OPPENHEIMER'S "Die Fermente").

[6] OPPENHEIMER: *Loc. cit.*, p. 220.

[7] LINTNER: Journal für praktische Chemie, 1887, xxxvi, p. 841.

[8] KJELDAHL: Comptes rendus des travaux du laboratoire de Carlsberg, 1879 (reference from EFFRONT'S "Enzymes and their Applications," p. 116).

that the salts of lead, zinc, and iron inhibit the action of diastase. Cole[1] has recently shown in his work on ptyalin that the negative ions stimulate while the positive ions inhibit the action of this enzyme. Cole's article was published since the work for this paper was completed. Jacobson[2] found that the splitting of hydrogen peroxide into water and oxygen, by the use of emulsion, or a watery extract of pancreas, was greatly retarded by the addition of the chlorides, nitrates, and sulphates of the alkalies, alkaline earths, and some heavy metals, while as a rule the addition of the salts of the organic acids had but little effect. In our own work[3] on the effect of ions on the decomposition of hydrogen peroxide by platinum black, we obtained results which may be explained by the assumption that in general the anions exert a stimulating action, and the cations a depressing action, so that the action of a given salt depends on whether the anion or cation is the more powerful. In this paper we have attempted to apply this theory to the enzymes of the pancreas.

I. The Effect of Ions on the Decomposition of Hydrogen Dioxide by a Watery Extract of Pancreas.

As a general rule all enzymes and catalytic agents have the power of splitting hydrogen peroxide into water and oxygen. If it be true, as is generally taught, that enzymes are catalyzers, we ought to expect the same results with the watery extract of pancreas on hydrogen peroxide as we found in the case of platinum black. We have done experiments to determine this.

Methods. — The extract was made from fresh hog's pancreas. The gland was finely minced after all fat had been removed. It was then mixed with sand and ground in a mortar to which a little water had been added. After being thoroughly ground more water was added to it, and it was filtered through cheesecloth. The vessel containing this extract was then placed in ice water. The bottles of hydrogen peroxide to be used were also kept in ice water. To measure the amount of oxygen given off, 50 c.c. eudiometer tubes, graduated in $\frac{1}{10}$ c.c., were used as the receiving vessels. The amount of oxygen given off was read and recorded at the end of two minutes and also five minutes. Wide-mouthed bottles of 100 c.c. capacity

[1] Cole: Journal of physiology, 1903, xxx, p. 202.

[2] Jacobson: Zeitschrift für physiologische Chemie, 1892, xvi, pp. 340–369.

[3] Neilson and Brown: This journal, 1904, x, pp. 225–228.

were used as vessels for the generating of the gas. Delivery tubes fitted in the corks of these bottles led to the eudiometer tubes. In each bottle was placed 25 c.c. of the solution to be tested. To this was added 5 c.c. of hydrogen peroxide and 2½ c.c. of the extract of pancreas. These bottles were shaken at intervals during the time the extract was acting, to insure the thorough mixing of the substance and to help free the oxygen from the liquid. Five bottles were used in each experiment. Controls, which contained 25 c.c. of distilled water in place of a salt solution, were made at short intervals. Five bottles were also used for each control experiment. In the tables the figures for the normal action of the pancreas are the averages of all the control experiments made, as the amount of oxygen freed in the different bottles in the controls varied but little. The results given in the table are a fair sample of those obtained from the numerous experiments performed.

a. **Effect of the cations.** — To test the effect of the positive ions, the chlorides of the alkalies, alkaline earths, and a few of the heavy metals were used. The concentrations employed were $\frac{n}{1}$, $\frac{n}{8}$, $\frac{n}{64}$, and $\frac{n}{512}$. The results are given in Table I.

TABLE I.

Solution used.	$\frac{n}{1}$ solution.		$\frac{n}{8}$ solution.		$\frac{n}{64}$ solution.		$\frac{n}{512}$ solution.	
	Cubic centimetres of oxygen set free in							
	2 min.	5 min.	2 min.	5 min.	2 min.	5 min.	2 min.	5 min.
Distilled water	14	33						
Potassium chloride	0	0½	2½	4½	7	13	12	32
Ammonium "	0	0	3	5	8	15	12	31
Lithium "	0	0	0½	2	4	8	12	32
Calcium "	0	0	2	4	7	14	11	30
Strontium "	0	0	1	3	14	32	11	29
Barium "	0½	1	1½	3	9	19	10	28
Magnesium "	0	0	3	5	9	20	9	28
Manganese "	..	..	5	7	6	15	11	28
Zinc "	..	..	1	4	5	13	16	32
Cobalt "	..	..	1	3	7	14	15	32
Aluminium "	0	0	0½	1	4	8	15	25

From Table I it is readily seen that in the $\frac{n}{1}$ solutions there is practically no splitting of the hydrogen peroxide, in the $\frac{n}{8}$ solutions the splitting is very small, in $\frac{n}{64}$ the splitting is but little more than half as much as in the controls, in the $\frac{n}{512}$ solutions the action of the extract approximates its normal action but never exceeds.

b. Effect of the anions. — To test the effect of the negative ions we used the sodium salts of a few inorganic and many organic acids. The concentrations used were the same as in the preceding table. The results obtained are seen in Table II.

TABLE II.

Solution used.	$\frac{n}{1}$ solution.		$\frac{n}{8}$ solution.		$\frac{n}{64}$ solution.		$\frac{n}{512}$ solution.	
	Cubic centimetres of oxygen set free in							
	2 min.	5 min.	2 min.	5 min.	2 min.	5 min.	2 min.	5 min.
Distilled water	14	33						
Sodium chloride	0½	2	1	3	7	15	14	33
" bromide	2	4	4	8	9	18	13	31
" nitrate	0	0	0	0¼	0	0½	4	7
" acetate	..	..	26	53	30	55	25	48
" formate	0	0¼	1	5	1	3	3	7
" valerianate	24	40	42	60	39	65	24	50
" butyrate	..	..	45	55	37	52	35	50
" chlorate	0½	1	0½	1	1	3	3	5
" fluoride	..	..	..	..	4	9	4	9
" sulphate	0	0	11	24	12	25	14	32
" tartrate	20	34	42	60	33	60	14	35
" oxalate	..	..	40	70	44	65	12	33
" succinate	..	..	31	60	32	55	16	36
" sulphite	0	0	19	35	12	27	13	34
" hyposulphite	25	30	30	60	20	42	14	33
" carbonate	..	..	50	..	50	..	40	70
" phosphate	..	..	40	70	55	..	20	35
" citrate	29	37	42	70	45	75	14	38

From Table II, it is noticed that the $\frac{n}{1}$ salts of acetic, valerianic, butyric, salicylic, tartaric, oxalic, succinic, phosphoric and citric acids cause marked acceleration, while those of hydrochloric, hydrobromic, nitric, formic, and hydriodic hinder the action. The hyposulphite accelerates in $\frac{n}{8}$ and $\frac{n}{64}$ solutions. The sulphate does not accelerate, but its inhibitory power is weak.

II. The Effect of Ions on the Hydrolysis of Butyric Ether by a Watery Extract of Pancreas.

It is a well-known fact that a watery extract of the pancreas hydrolyzes butyric ether. The object of this part of the paper is to show that the hydrolysis of butyric ether is affected by salt solutions in the same manner as the decomposition of hydrogen dioxide by a watery extract of pancreas.

Methods. — The extract of pancreas was made in the same manner as in Part I. Butyric ether in presence of watery extract of pancreas splits into alcohol and butyric acid. The amount of acid produced is the indicator of the activity of the pancreatic extract. This was determined by titration with $\frac{n}{20}$ sodium hydrate, with phenolphthalein as an indicator.

The butyric ether and the extract were placed in tightly corked test tubes, which were kept in an incubator at 40° C. for one hour, during which time they were shaken several times. After removal from the incubator they were placed on ice to stop the action, and then titrated. The following tubes were used:

1st. Tube A contained 5 c.c. of distilled water, and 0.2 c.c. of concentrated butyric ether. This was to determine the acidity of the butyric ether. Tube B contained 5 c.c. of distilled water, 0.2 c.c. of butyric ether, and $2\frac{1}{2}$ c.c. of the pancreas extract. The acidity of tube B minus the acidity of tube A gives the normal action of the extract.

2d. Tube A contained 5 c.c. of the salt solution to be tested and 0.2 c.c. of butyric ether. This gives the acidity of the butyric ether and the salt. Tube B contained 5 c.c. of the same salt as tube A, 0.2 c.c. of butyric ether, and $2\frac{1}{2}$ c.c. of pancreatic extract. The acidity of tube B minus that of tube A gives the action of the extract under the influence of the salt. By comparing 1 and 2 it is readily seen whether the salt used has an influence on the action of the pancreatic extract.

a. **Effect of positive ions.** — The solutions and the concentrations used are practically the same as those in A of Part I, as will be seen with the results in Table III.

TABLE III.

Solution used.	Amount of butyric acid in terms of $\frac{n}{20}$ sodium hydrate.				
	$\frac{n}{1}$ solution.	$\frac{n}{32}$ solution.	$\frac{n}{128}$ solution.	$\frac{n}{256}$ solution.	$\frac{n}{512}$ solution.
Distilled water . .	6.0				
Potassium chloride	2.4	4.6	5.1	5.2	5.6
Lithium “	2.2	3.2	4.2	4.8	4.3
Cæsium “	4.3	5.2	5.8	5.4	6.0
Calcium “	1.8	4.6	4.9	5.1	5.5
Strontium “	2.0	5.1	5.0	5.4	5.7
Barium “	2.0	5.0	5.7	6.4	6.1
Magnesium “	1.7	3.0	4.2	6.4	5.8
Manganese “	..	4.1	5.4	4.8	5.3
Cobalt “	..	4.2	5.7	5.8	5.4
Zinc “	..	3.16	5.1	4.6	4.8
Aluminium “	..	2.04	3.1	5.0	5.5

The salt solutions of Table III in the $\frac{n}{1}$ concentrations inhibit very markedly the hydrolysis of butyric ether by a watery extract of pancreas. This inhibition decreases as the concentration decreases until in the $\frac{n}{256}$ solutions barium and magnesium allow about normal action, while the other solutions have slight inhibitory effects. In the $\frac{n}{512}$ concentrations the amount of hydrolysis was about the same as in the distilled water. Solutions as dilute as $\frac{n}{2048}$ were used with results about the same as if they had been distilled water.

b. **Effect of negative ions.** — The solutions, concentrations, and their effects will be seen in Table IV.

TABLE IV.

Solution used.	Amount of butyric acid formed, in terms of c.c. of $\frac{n}{20}$ sodium hydrate.						
	$\frac{n}{1}$	$\frac{n}{8}$	$\frac{n}{32}$	$\frac{n}{64}$	$\frac{n}{128}$	$\frac{n}{256}$	$\frac{n}{512}$
Distilled water . . .	6.0						
Sodium chloride . .	4.0	3.35	5.02	. .	4.0	5.0	5.7
" bromide . .	3.0	4.14	4.3	. .	5.0	5.2	5.7
" iodide . . .	3.4	4.1	5.12	3.02	5.0	5.1	5.4
" nitrate . . .	4.0	3.88	2.48	5.5	5.5	5.6	5.7
" hyposulphite	. .	3.7	4.65	5.2	5.0	5.1	5.3
" chlorate . .	3.0	6.3	5.07	6.03	5.0	5.6	5.4
" succinate . .	8.6	8.4	9.15	9.86	7.65	6.0	5.4
" acetate . .	2.7	10.6	11.6	8.1	7.8	7.4	6.8
" sulphite . .	. .	3.52	4.7	5.72	5.2	5.8	6.0
" carbonate .	. .	. .	7.0	7.8	8.8	9.0	6.9
" fluoride . .	. .	2.2	2.4	2.48	2.2	2.65	3.5
" sulphate . .	. .	5.7	6.45	5.7	5.3	5.5	6.0
" tartrate . .	11.74	8.05	8.0	6.4	6.55	6.45	7.0
" salicylate . .	1.66	3.05	5.2	5.8	5.5	5.4	5.0
" phosphate .	. .	. .	8.07	8.67	8.42	6.6	5.9
" citrate . . .	9.9	10.92	7.92	7.2	7.1	6.4	6.6
" valerianate .	9.52	9.46	9.48	9.0	8.0	7.5	6.0
" oxalate . .	. .	8.0	6.46	6.0	6.5	6.1	6.4
" formate . .	6.5	7.7	7.05	7.0	6.8	6.0	5.8

From Table IV, it will be noticed that there is some inhibition in a few cases, but as a rule the amount of acid formed in presence of the optimum concentration of the salts recorded is greater than in distilled water. In the $\frac{n}{1}$ concentrations, except with the succinate, tartrate, citrate, valerianate, and formate, there was more or less inhibitory effect. Not all of the solutions were used in the $\frac{n}{1}$ concen-

tration, as there was an appreciable amount of acid or alkali present in some of them. As asserted by Kastle and Loevenhart, the fluoride inhibits even in very weak solutions. $\frac{n}{4000}$ concentration of fluoride was found to have a slight inhibitory action. The chloride, bromide, iodide, nitrate, sulphite, hyposulphite, and salicylate of sodium were found to have no accelerating action. The inhibitory action of the above mentioned solutions decreased gradually from $\frac{n}{1}$ concentration to the $\frac{n}{512}$, where the action was about the same as that in distilled water. The salts which accelerate do not have their optimum action at the same concentration. The amount of the hydrolysis in the $\frac{n}{1}$ concentration of the sodium tartrate is practically twice that in distilled water, while the weaker concentrations do not accelerate so much.

The $\frac{n}{32}$ concentration of the sodium acetate acts about the same as the $\frac{n}{1}$ concentration of sodium tartrate, while the other concentrations of this salt do not cause as great hydrolysis.

III. Effect of the Anion, when combined with Potassium and Lithium, on the Hydrolysis of Butyric Ether by a Watery Extract of Pancreas.

A few potassium and lithium salts were used in order to compare more fully the effect of the anion when combined with sodium, potassium, and lithium. The solutions and concentrations employed and the results are seen in Tables V and VI.

TABLE V.

Solution used.	Amount of butyric acid formed in terms of c.c. of $\frac{n}{20}$ sodium hydrate.			
	$\frac{n}{1}$	$\frac{n}{8}$	$\frac{n}{64}$	$\frac{n}{512}$
Distilled water . . .	11.0			
Potassium bromide .	5.3	7.3	9.0	11.0
" acetate . .	..	9.2	13.6	13.0
" sulphate .	6.0	7.1	6.1	6.4
" tartrate . .	8.9	12.6	13.5	12.5
" oxalate . .	8.9	13.0	12.6	12.0
" citrate . .	14.0	10.5	10.0	11.0

TABLE VI.

Solution used.	Amount of butyric acid formed in terms of c.c. of $\frac{n}{20}$ sodium hydroxide.			
	$\frac{n}{1}$	$\frac{n}{8}$	$\frac{n}{64}$	$\frac{n}{512}$
Distilled water . . .	10.0			
Lithium bromide . .	..	6.6	9.3	10.0
" acetate . . .	10.5	11.0	11.0	11.3
" salicylate . .	..	5.25	9.0	9.8
" sulphate . .	6.0	8.05	10.2	11.0
" oxalate . . .	..	9.0	10.9	11.0
" tartrate . . .	..	10.0	10.8	9.8
" citrate . . .	..	10.0	11.0	11.0

Since the potassium and lithium ions have a greater depressing action than the sodium ion, it is obvious that in order to get the greatest stimulating effect of any anion (as citrate, tartrate, valerianate, etc.), the sodium salt should be used in preference to potassium or lithium. This fact is clearly demonstrated by the results of Tables V and VI.

Summary.

By comparison of Tables I and III, it is seen that there is a constant inhibition by the chlorides of the alkalies, alkaline earths, and heavy metals used, on both the decomposition of hydrogen peroxide and the hydrolysis of butyric ether by pancreas extract. Moreover, it will be noticed that the depressing effect is much more marked on the decomposition of hydrogen peroxide than on the hydrolysis of butyric ether. The explanation for this may be that the hydrogen peroxide is readily soluble in the water and the salt used, while the butyric ether is much less soluble. The contact of the catalytic agent in the extract with the salt and the hydrogen peroxide is better than where the butyric ether was used. By comparison of Tables II and IV there are seen to be differences in the action of certain salts on the decomposition of hydrogen dioxide and the hydrolysis of butyric ether, by a watery extract of pancreas. The nitrate and chlorate of sodium inhibit strongly the decomposition of hydrogen dioxide, while

they inhibit only moderately the hydrolysis of butyric ether. The sodium formate inhibits strongly in the first case, but accelerates slightly in the latter, while the sodium salicylate acts in just the reverse manner, accelerating slightly the hydrogen dioxide decomposition, and depressing slightly the butyric ether hydrolysis. But in general, the salt in an optimum concentration which accelerates in one case does likewise in the other; the most marked accelerant in one case being also the most marked accelerant in the other.

Our results can be explained on the assumption that, in general, in the decomposition of hydrogen peroxide and the hydrolysis of butyric ether by a watery extract of pancreas, the cations have a depressing or retarding action and the anions have an accelerating action.

Our thanks are due Professor Stewart and Dr. Lyon for criticisms.

DOES AN ANTAGONISM EXIST BETWEEN ALKALOIDS AND SALTS?

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I. Introduction.

IN 1899 Loeb[1] showed that the gastrocnemius muscles of frogs may be made to beat rhythmically when immersed in pure solutions of certain sodium, lithium, cæsium, or rubidium salts, but that the rhythmical contractions cease when a small amount of any soluble calcium salt is added to these solutions. A little later he showed that the same facts hold true for the rhythmical contractions of the hydromedusa, Gonionemus.[2] If the nerve ring is severed from this animal by a transverse cut, the piece containing the ganglia will continue to beat in sea water, but the muscular umbrella will not. In a pure sodium chloride solution, however, the umbrella, deprived of its nervous connections, will also continue to beat. The rhythmical contractions of either piece are inhibited or stopped upon the addition of calcium to the solutions in which they lie, but much less calcium is required to bring the (myogenic) contractions of the umbrella to a standstill than is necessary to stop the (neurogenic) contractions of the ring containing the ganglia. Lingle[3] demonstrated the effect of calcium in inhibiting and stopping muscular contractions in his experiments on heart muscle.

A long series of experiments by Loeb[4] showed that when the gastrocnemius muscles of frogs are immersed in solutions of certain salts whose anions are liable to form insoluble calcium compounds they become exceedingly sensitive and show a form of irritability (contact irritability?) which they do not show ordinarily. This irritability and the muscular twitchings which result when the muscle

[1] Loeb, J.: Festschrift für Professor Fick, Braunschweig, 1899.
[2] Loeb, J.: This journal, 1900, iii, p. 383.
[3] Lingle, D. J.: This journal, 1901, iv, p. 276.
[4] Loeb, J.: This journal, 1901, v, p. 362.

is removed from these solutions to air (or certain other media) are done away with when the muscles are allowed to remain for some time in solutions containing calcium ions. If the nerve alone is put into the solution of a salt whose anion is liable to form an insoluble calcium compound, the muscle begins to twitch in about five minutes and finally goes into tetanus. When the nerve is taken out of this solution the contractions cease. Yet it can be shown that the solution does not stimulate the nerve directly, but only increases its irritability, for when the same nerve is brought in contact with any other aqueous or solid body the contractions recommence; nor do they cease until the nerve is again surrounded by air on all sides. This modified or increased irritability of the nerve is also done away with when the nerve is immersed in a solution containing calcium ions.

Attention was also called by Loeb to the fact that those salts which modify or increase the irritability of muscle and nerve are identical with those which constitute the group of the so-called saline cathartics. He further expressed the idea that the medicinal effects of the saline cathartics probably lay in their power of increasing the irritability of the nervous or muscular elements in the intestine. J. B. MacCallum [1] has recently proved the correctness of this idea by his experiments on rabbits. The injection of barium chloride, sodium citrate, sodium fluoride, sodium sulphate, sodium tartrate, sodium oxalate, sodium phosphate, or magnesium sulphate intravenously or subcutaneously, or their direct application to the intestinal wall brings about an increased peristalsis of the intestines. The increased peristaltic movements are promptly inhibited by the subsequent injection of a small amount of calcium chloride solution.

Loeb [2] also succeeded in bringing about a hypersensitiveness of the skin in frogs by dipping their feet for a short time into solutions of sodium citrate, oxalate, sulphate, carbonate, or phosphate. Frogs treated in this way are as sensitive to contact with pure water as they are naturally to strong acids.

In all the experiments cited above the changes in irritability were brought about by altering the quotient $\frac{\textit{Concentration Na ions}}{\textit{Concentration Ca ions}}$ in the tissues. When in the gastrocnemius muscle of a frog, for example,

[1] MacCallum, J. B.: University of California Publications, Physiology, 1903, i, p. 5; This journal, 1903, x, p. 101.

[2] Loeb, J.: Decennial Publications, University of Chicago, 1902, x.

the value of the denominator was decreased (while that of the numerator was increased) through immersion of the muscle in an isotonic sodium citrate solution, the irritability of the muscle was increased, so that when brought in contact with air (or certain other substances) the muscle twitched or even went into tetanus. The contractions of the muscle ceased, however, when the value of the denominator was again increased.

II. Does an Antagonism exist between Strychnine and Calcium Chloride?

The question now arose whether an increase in irritability when brought about by some other means than by electrolytes can also be inhibited by calcium ions. The poisonous effects of strychnine evidence themselves, as is well known, by increased reflex irritability. In order to see whether calcium ions have the power of inhibiting this effect I undertook, at the suggestion of Dr. Loeb, the following experiments on rabbits and frogs.

The experiments on rabbits were performed in the following way. In each experiment two rabbits were chosen as nearly as possible of the same breed, weight, and physical condition. While one was injected with strychnine only, the other was injected with the same amount of strychnine plus calcium chloride. The nitrate of the alkaloid was employed dissolved in a small amount of boiled distilled water, while the calcium chloride was given in a $\frac{1}{8}$ molecular solution.

Both solutions were injected into one of the veins of the rabbit's ear. Whenever injected separately the strychnine was injected into one ear while the calcium was injected into the other. Eleven experiments were made in all. In two of them the alkaloid was given first and then the calcium chloride. In two others the strychnine and calcium chloride were mixed before injecting them. In the remaining seven experiments the calcium chloride solution was given first, and this was followed by the strychnine. The strychnine was at times given in one large dose, but usually in small, divided doses until its first physiological effects made themselves felt, when the injections were stopped. From 5 to 25 c.c. of the $\frac{1}{8}$ molecular calcium chloride solution were injected in divided doses in the attempt to antagonize the action of the strychnine. I convinced myself by proper preliminary experiments that the injections of calcium

chloride, in the quantity and concentration used in these experiments, were not fatal in themselves. Whenever the strychnine was injected after the calcium chloride, an interval of five to thirty minutes was allowed before the strychnine was injected, so that the calcium chloride was given time to circulate through the body. After the solutions were injected the animals were allowed to lie quietly on the floor. External stimuli such as draughts, sounds, etc. were shut out as far as possible, and both the control animals and those experimented upon were treated alike in every detail.

The results of the eleven experiments may be given briefly as follows. In three experiments the animals injected with strychnine plus calcium chloride recovered, while those injected with strychnine only, died. In three other experiments the reverse was the case, and the control animals which had received only strychnine recovered, while the others died. In the remaining five experiments both the control and the experimental animals died. In four of the experiments of this series of five, the animals which had received both calcium chloride and strychnine died sooner than those which had received strychnine only. In the remaining experiment of this series the reverse was the case.

We see therefore, when we review the results of the entire set of eleven experiments, that the rabbits which received calcium chloride in addition to the strychnine fared no better than those which received strychnine only. *This seems to justify the conclusion that calcium chloride cannot counteract the effects of strychnine.*

In warm-blooded animals poisoned with strychnine, sudden death due to respiratory failure is exceedingly likely to occur. On this ground some objection could be raised to the experiments on rabbits in that it might be urged that the rabbits died before the calcium chloride had an opportunity to act. For this reason it seemed best to make a series of experiments on frogs, for since respiration is carried on so largely through the skin in these animals, sudden death is not apt to occur. Frogs at ordinary temperature stand large doses of strychnine, remain in absolute tetanus for days at a time and still recover.

The experiments on frogs were carried out in a way similar to those on rabbits. I made eight series of experiments in all. In each series, six to twelve frogs were chosen of as nearly as possible the same size, and these were divided into two lots, one of which was injected with strychnine only, while the other was injected with strych-

nine plus calcium chloride. The frogs were kept on moist towels under separate funnels, and the whole supported on a wire screen. The towels were irrigated with water from time to time, or changed entirely, so that the strychnine excreted by the frogs could not be reabsorbed through the skin. The amount of strychnine injected into each frog varied from 0.2 mgm. to 4 mgms. I usually used 0.8 mgm., which I found just sufficient to cause the majority of the frogs to go into opisthotonos. The calcium chloride was injected as a $\frac{1}{8}$ molecular solution, the amount varying from 0.4 c.c. to 1.7 c.c. Although the latter amount is not fatal for normal frogs, it is likely to prove so in frogs injected with strychnine. For this reason I always gave quantities above 1 c.c. in divided doses, several hours elapsing between successive doses. In a few experiments I gave as much as 8 c.c. in divided doses. In those frogs which received both strychnine and calcium, the two substances were usually injected separately, the one into the ventral lymph sac, the other into the dorsal lymph sac. Ordinarily calcium chloride was given first, and then the strychnine five minutes to two hours later. Sometimes the order was reversed, and in a few experiments both were given simultaneously. The order in which the solutions were injected did not alter the outcome of the experiments.

Without detailing the separate experiments it may be said that forty-six frogs were experimented upon, of which one half received strychnine only, while the other half received strychnine plus calcium chloride.

When small doses of calcium chloride were given those frogs which received calcium chloride in addition to the strychnine fared no better than those which received strychnine only. The onset of the symptoms of strychnine poisoning, their severity, the rate of recovery, and the mortality among the frogs injected with strychnine plus calcium chloride were no different from those of the frogs injected with strychnine alone. Larger doses of calcium chloride have a marked "depressing" effect upon the frogs. After such doses the frogs lie flat upon the table with their legs adducted and do not struggle or try to escape when touched, as do normal frogs. When frogs which show these effects of the calcium chloride receive an injection of strychnine, they become hypersensitive and go into opisthotonos a little later than those which have received strychnine only. Several times *after very large and repeated doses of calcium chloride* I have seen *frogs injected with strychnine show no hypersensitiveness whatso-*

ever, although the control frogs injected with strychnine alone went into opisthotonos. After such amounts of calcium chloride, however, as are large enough to cause any difference at all in the symptoms of strychnine poisoning, *the mortality is invariably higher among the frogs injected with strychnine plus calcium chloride than among those injected with strychnine only.*

III. Does Sodium Citrate, Magnesium Chloride, or Barium Chloride influence the Poisonous Effects of Strychnine?

Since immersion of a muscle in sodium citrate increases its irritability, the question arose: Does the injection of sodium citrate increase the susceptibility of animals to strychnine?

Experiments were performed on six rabbits and twelve frogs in a way similar to that already described under II. *No difference was noted in the time of onset of the first symptoms, in the severity of the symptoms, in the rate of recovery, or in the mortality between those animals injected with strychnine only and those injected with strychnine plus sodium citrate.* Very large doses of sodium citrate were able to some extent to *retard* the appearance of the first symptoms of strychnine poisoning in frogs, but the amounts required to accomplish this often, as in the case of calcium chloride, ended the life of the animal.

I tried to see also whether the toxic effects of strychnine could be counteracted by barium chloride, but the poisonous effects of the latter seemed, in the three experiments I tried on rabbits, only to add themselves to those of the strychnine and so hasten the death of the animal.

In two experiments on rabbits magnesium chloride was also found to be ineffective in antagonizing the poisonous action of strychnine.

Strychnine was the alkaloid chosen in these experiments, as it of all the alkaloids seemed best adapted for the study of the question as to whether an antagonism exists between alkaloids and certain salts. The pharmacological effects of strychnine are suggestive of the effects produced by solutions of certain sodium salts whose anions are liable to precipitate calcium. As calcium chloride is able to counteract the effects of these sodium salts, it seemed reasonable to expect that if an antagonism did exist between alkaloids and salts, calcium chloride should be able to counteract the effects of strychnine also. This, however, was not found to be the case, as the experiments described

above indicate. This does not mean, of course, that no antagonism exists between any alkaloid and certain salts, but it points in that direction. I intend to continue these experiments with other alkaloids than strychnine in the effort to obtain further data concerning this question.

THE SIMULTANEOUS ACTION OF PILOCARPINE AND ATROPINE ON THE DEVELOPING EMBRYOS OF THE SEA-URCHIN AND STARFISH. — A CONTRIBUTION TO THE STUDY OF THE ANTAGONISTIC ACTION OF POISONS.

By TORALD SOLLMANN.

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I. Introduction.

THE simultaneous action of two poisons producing opposite effects offers some very interesting problems, both from a theoretical and from a practical standpoint. Many instances are known and utilized in which the stimulant or depressant action of a poison is removed by another substance having an opposite action if used alone; the two "neutralizing" each other. This may be termed "functional antagonism" to distinguish it from chemical antagonism; the removal of the action in the latter case being due to the production of inactive compounds by the chemical combination of the two substances.

Whilst the functional antagonism consists in many cases in an antagonistic action on different structures (one substance, for instance, depressing the ganglia whilst another stimulates the endings, etc.), there are also some instances in which the antagonism undoubtedly takes place in the same cell, for instance, in monocellular organisms, or those in which all the cells have equivalent functions. There is also reason to suppose that antagonism may occur in a single constituent or structural or functional element of a cell; but this point is difficult to prove. Granting its possibility, we are confronted by the question of the nature of this antagonism. When a stimulant and depressant, acting together, produce no apparent effect, is there in reality no action? Or do both drugs produce their effects as if they were present alone, the two opposed actions merely obscuring and hiding each other? The latter would seem,

a priori, the more likely explanation; but its experimental proof has not been furnished.

A decision between the two views is of considerable practical importance. For if we can actually restore a poisoned cell to its normal condition by administering an antidote, the treatment of poisoning becomes a comparatively simple matter; and so does the treatment of disease in its corresponding phases. If, on the other hand, the two opposed actions merely obscure each other, we may for a time be unable to affect the particular function observed; but the cell will not be normal. The simultaneous action of these poisons — we may say, their simultaneous combination with the biogen — must result in alterations of this biogen; in chemical, functional, and structural changes. In most cases the biogen will probably be injured, so that it will be more readily fatigued, and less resistant to poisons; it will therefore behave differently in the early and late stages of the actions of the two poisons; or if their absolute quantity is altered, even when the same relative ratio is maintained. If the biogen is injured, the depressant action would become more prominent as the action is prolonged, or as the doses are increased. In such a case, it is evident that the treatment of poisoning is not simple: the final injury to the cell *may* be even greater if the antidote is given than if it is omitted. This point would need to be studied in each particular instance, although some typical examples might permit generalizations.

The detailed investigation of all forms of antagonism would therefore be desirable. From the theoretical standpoint, however, it would be important to simplify the problem by choosing two poisons having opposite actions confined to the same structure. This ideal condition can be most nearly realized by employing simple organisms in which one important, readily observable function predominates. The gas-formation by yeast appeared to furnish a suitable object for this study. This was investigated by H. D. Haskins [1] at this laboratory. He failed to find any drug which would increase the gas formation; this increase was obtained, however, by heating to about 37° C. Many poisons were found to lessen the formation of gas. Of these strychnine was selected, and the gas formation was observed in pure glucose solution, and in solutions containing strychnine, both sets of tubes being exposed to varying temperatures. It was found that the curves for the pure solution and for the poisoned solution did not run parallel. The strychnine curve was zigzag, showing

[1] Haskins, H. D.: American journal of the medical sciences, 1903, cxxvi, p. 1036.

that the depression was greater at certain temperatures than at others. The irregularity indicates that heat and strychnine do not actually neutralize each other; and that, therefore, an antagonistic action by the mutual obscuring of the effects exists. It is, however, possible that the mechanism of the action of strychnine and heat are quite different ; so that conclusions as to the antagonism of drugs should not be drawn from this example.

A. P. Mathews [1] has observed that the development of the embryos of starfish and sea-urchins is hastened by pilocarpine and retarded by atropine. This observation pointed to more suitable material, and I accordingly undertook the present research at the Marine Biological Laboratory of Wood's Hole. In simple organisms, such as the embryos of starfish and sea-urchins the function of growth is peculiarly predominant and is easily observed. The fact that the action of large doses of pilocarpine is indistinguishable from that of atropine also points to the conclusion that the stimulation involves the identical structures which are depressed by atropine. The combined action of the two drugs indicates this still more strongly, as we shall see.

II. Methods.

The methods employed by Mathews were also used by me, with a few very insignificant modifications of detail. In the earlier experiments the specimens were actually measured. But as all the embryos in a sample were rarely developed to the same degree, it seemed impossible to obtain representative measurements; and it was found better to transfer a large number of eggs to Minot dishes, comparing the eggs under a low power with the other samples which stood nearest to them in the scale of development. Deformities and differences in activity could in this way be taken into account, as well as differences in size. Variations which could not be detected by this method could safely be omitted, as lying within the limits of error. To obviate variations due to differences of light, heat, etc., two series were usually made at the same time; each comprised control samples in pure sea-water and samples with varying quantities of pilocarpine, of atropine, and of both together. The results were then represented as curves, from which the conclusions are deduced.

[1] Mathews, A. P.: This journal, 1902, vi, p. 207.

It may be mentioned that the drugs had usually no effect during the first day. No attempt was made to investigate whether this is due to a very slow penetration of the poisons, or to an insusceptibility of the early embryos.

III. Results.

The effects of the poisons were very similar on both Arbacia (Experiments I to III) and Asterias (Experiments VI to IX). Considerable difference existed, however, in the susceptibility of the various lots of eggs; and even in the same lot, some eggs were affected much more than others.

A. Single drugs. 1. *The effect of pilocarpine on development.* — Pilocarpine hydrochloride, added to sea-water in concentrations of 0.2 to 2.0 : 10000 generally hastens development, and increases the size of the embryos. The effect becomes apparent only after a day. If any stimulation occurs, it is marked with 0.2 : 10000, and increases with larger doses, to an optimum lying between 0.2 and 1.0 : 10000. The optimum concentration is the lower, the longer the embryos have been exposed to the pilocarpine. With concentrations slightly larger than the optimum the development approaches the normal (0.5 to 1.0); and with still larger doses it is less than normal (0.2 to 2.0); here also, the doses necessary to produce depression lessen with the time of exposure, the optimum doses in the early days of the experiment becoming depressant later on. In some samples pilocarpine produced no stimulation, concentrations up to 1.0 producing no effect, while larger doses caused depression.

The stimulant action of pilocarpine was great when the normal development was rapid (Experiments I and VII); but was small with poor development (Experiments II, VIII, and IX). Only in Experiment III was there a good development, with small pilocarpine effect. The rate of development did not, however, seem to influence the optimum concentration of pilocarpine.

Large doses of pilocarpine hastened the death of the embryos; smaller doses had little effect; in no case was death delayed by the drug.

Mathews (*loc. cit.*) had similar results; concentrations of 0.15 to 1.0 : 10000 had sometimes no effect; the optimum concentration lay between 0.25 and 0.5 : 10000.

2. *The effect of atropine on development.* — No stimulation was observed with any concentrations. 0.1 and 0.2 : 10000 of atropine sulphate generally had no effect, but in some cases even these concentrations depressed the development. Concentrations of 0.3 and upward caused marked retardation in practically all cases, the depression increasing with the concentration. In a very few instances was 0.5 without effect. The depressant action increased with the time during which the drug acted; concentrations which were without effect on the first days of the experiment becoming depressant on the later days. The depressant action was not influenced perceptibly by the normal rate of development. The depressant action of atropine was usually large when the pilocarpine stimulation was small (Experiments II, III, and VIII); but with a large pilocarpine stimulation, the atropine depression may be either large or small.

Concentrations of atropine between 0.1 and 0.2 : 10000 have no influence on the death of the embryos; higher concentrations usually hasten death, but may have no effect upon it.

The results agree with those of Mathews, who found that concentrations of 0.1 to 1.0 : 10000 retarded growth in proportion to the concentration.

B. The effects of the simultaneous action of both drugs. 3. *The results when atropine alone is depressant, pilocarpine stimulant.* — In Experiments I, 4 and 5,[1] II, 5, VII, 1 to 4, atropine 0.5 : 10000 abolishes the stimulation of 1 : 10000 pilocarpine, reducing the development below the normal control; but the embryos develop better than with the atropine alone. In VIII, $\frac{3}{4}$, atropine 0.3 : 10000 abolishes the stimulation of 0.2 pilocarpine, the development being retarded as with atropine alone. In II, 5, death is hastened.

The addition of depressant concentrations of atropine not only abolishes the pilocarpine stimulation, but the development is even less than that of control specimens to which no drugs have been added; death is also hastened. The development is, however, generally better than if atropine had been used alone; so that the pilocarpine stimulation partially neutralizes the atropine depression. The early death, produced by atropine, is not usually delayed.

[1] Fourth and fifth days (the arabic numerals following the experiment number, always refer to the days during which the drugs have acted).

4. *The results when atropine alone is depressant, pilocarpine inactive.* — In Experiments II, 4, VI, 2, and VII, 6, atropine 0.5 : 10000 used with 1 : 10000 pilocarpine causes a greater depression than atropine used alone. In VII, $\frac{3}{4}$, 0.3 : 10000 atropine with 0.5 : 10000 pilocarpine causes the same depression as atropine alone.

Depressant concentration of atropine (0.5 : 10000) causes 1 : 10000 pilocarpine to become depressant, if the latter was inactive when used alone: the depression produced by both drugs being greater than that caused by atropine alone.

5. *Results when both atropine and pilocarpine, used separately, are depressant.* — In Experiments II, 6 and 7, III, 3 and 4, and VI, 4, the combination of 0.5 : 10000 atropine with 1 : 10000 pilocarpine produces greater depression and quicker death than either alone. In VIII, $\frac{3}{4}$, 0.3 : 10000 atropine with 1 : 10000 pilocarpine produces the same depression as atropine alone.

The combination of depressant concentrations of atropine and pilocarpine produces greater depression and quicker death than either used alone.

6. *Results when atropine alone is inactive, pilocarpine stimulant.* — In Experiments I, 1 to 3, and VI, 1, atropine 0.5 : 10000 abolishes the stimulation produced by pilocarpine 1 : 10000, the development being the same as that of specimens to which no drug was added. In VI, 1, 0.1 : 10000 atropine has no effect on the stimulation produced by 1 : 10000 pilocarpine. In VIII, $\frac{3}{4}$, it abolishes the stimulation produced by 0.2 : 10000 pilocarpine.

Concentrations of atropine which have no effect when used alone, nevertheless abolish the stimulant action of pilocarpine.

7. *Results when both atropine and pilocarpine are ineffective if used separately.* — No effect is produced with atropine 0.1, 0.3, and 0.5 : 10000, and pilocarpine 0.2, 0.5, and 1 : 10000 in Experiment II, $2\frac{1}{2}$; with atropine 0.1[1] and pilocarpine 1[1] in II, 4, III, 2, and VII, 2; with atropine 0.1 and pilocarpine 0.5 in VIII, $\frac{3}{4}$; with atropine 0.1 and 0.3 and pilocarpine 0.2, 0.5, and 1 in IX, 1; with atropine 0.1 and pilocarpine 0.2, 0.5, and 1 in IX, 2; and with atropine 0.3 and pilocarpine 0.2 in IX, 2. Only in IX, 2, is there some depression when 0.3 atropine is combined with 0.5 and 1 pilocarpine.

[1] : 10000.

When either alkaloid is ineffective when used alone, there is also no result when the two are exhibited together, in the large majority of cases; if any result is produced, it is depressant.

8. *Result when atropine alone is inactive, pilocarpine depressant.* — In Experiments III, 3 and 4, VI, 4, and VIII, $\frac{3}{4}$, the addition of 0.1 : 10000 atropine increases the depressant action of 1 : 10000 pilocarpine. In II, 6 and 7, the result is doubtful.

The addition of inactive concentrations of atropine (0.1 : 10000) to depressant doses of pilocarpine (1 : 10000) increases the depressant action of the latter.

9. *The effect of atropine on the optimum stimulant concentration of pilocarpine.* — The addition of 0.5 atropine always lowers the optimum concentration of pilocarpine, both minimal and maximal, on observations comprising four experiments, and twelve separate days. This reduction is quite marked, the optimum being reduced from a mean of 0.5 to 1.5 : 10000 when pilocarpine is used alone, to a mean of 0–0.5 : 10000 when used with 0.5 : 10000 atropine.

When lower concentrations of atropine are used, the result is not so great, and hence less constant:

In Experiment IX, $\frac{3}{4}$, the optimum of 0.3 was increased to 0.5 by 0.3 : 10000 atropine. In Experiment VIII, 2, the optimum of 0–1.0 was reduced by 0.3 atropine to 0–0.2.

IV. Discussion of the Results.

The typical *action of pilocarpine* may be represented by the curves *a*, *b*, *c*, and *d* of Figure 1, according to the length of time during which

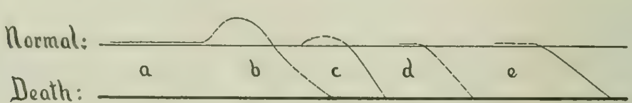

Figure 1. — Sequence of pilocarpine action. ——— represents the normal development; – – –, the development under the influence of pilocarpine; ▬▬, the level at which death occurs. The concentration in each curve increases from left to right; *a* is the effect during the first day, *b* and *c* during the succeeding days, *d* at the end of the experiment.

it has acted. Fig. 1, *e* shows an atypical result, sometimes seen, in which the stimulant phase is lacking.

Fig. 2, *a*, *b*, and *c* show the *action of atropine* (. . . . . .) during

successive periods; the atypical form, in which ordinary doses produce no depression, may be represented by 2, *d*.

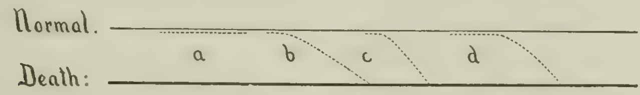

FIGURE 2.—Sequence of atropine action. *a*, *b*, and *c* show the action of atropine (......) during successive periods; the atypical form, in which ordinary doses produce no depression, may be represented by II, *d*.

The combination of the typical actions is seen in Fig. 3; of the atypical actions in Fig. 4.

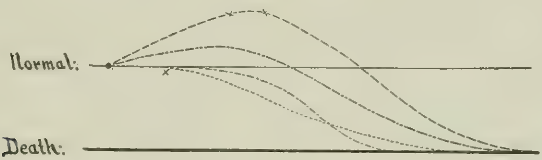

FIGURE 3.—Combined action of pilocarpine and atropine. Comparison of typical curves. See legend of Fig. 4.

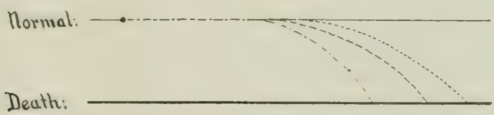

FIGURE 4.—Combined action of pilocarpine and atropine. Comparison of atypical curves. ——, normal development; – – –, development under pilocarpine; ......, under atropine; ––..––..––, mathematical average of the action of both drugs; –.–.–, observed resultant; ▬▬, death point; × to ×, region of optimum pilocarpine stimulation.

It is seen that the observed resultant is always less than the mathematical average (indicating greater depression). It is also to be remarked that doses of atropine, too small to produce any action alone, are nevertheless sufficient to remove the pilocarpine stimulation, or to convert it into an actual depression. Similarly, doses of pilocarpine which are inactive alone, increase the depression when used with atropine. In other words, the combined action of the two drugs renders the protoplasm more subject to depression. The observations show conclusively that *both drugs are acting, even when they are antagonistic.* The only case in which no action results is when both drugs, used separately, are inactive.

The fact that the depressant action tends to predominate — that it is much easier to remove a stimulation than a depression — is generally accepted; we have now given its explanation. It is also seen that a stimulant may counteract a depression, even when both act upon the same cell and presumably on the same structure. In this sense stimulation is a useful therapeutic measure. However, the depression lessens the stimulation, relatively as well as absolutely: the stimulant does not produce as great a rise of the depressed curve as of the normal curve. The depression even tends to reverse the stimulation into a depression, causing a fall in the curve. The dose which produces the maximum of stimulation in a normal organism may therefore produce an additional depression in a depressed organism, and the optimum concentration for the latter lies therefore always lower than in the normal condition. The practical rule follows that *stimulants should be given in smaller doses during depression than when the cell is normal.* This is very important, for the ordinary idea is that a larger dose is more useful. The experiments show that the larger dose may even be harmful. Similar conclusions were drawn by Stokvis[1] concerning the antagonism of poisons on the frog's heart: the best results were obtained when medium doses of the poison were antagonized by dilute solutions of the antidote. These results apply only to depression produced by poisons which act on the same cells. It is conceivable that the conditions are different when the antagonism is obtained by the stimulation of another structure, for instance, when a peripheral depression is to be relieved by a central stimulation.

V. Conclusions.

1. Small doses of pilocarpine hasten the development of the embryos. Larger doses, or a prolongation of the action, produce the opposite result.

2. Small doses of atropine have no action. Larger doses hinder development.

3. When both drugs are used, there may be antagonism or synergism, according to the conditions; but the depressant action always tends to predominate.

4. The observed resultant is always below the mathematical average.

[1] Stokvis, B. J.: Virchow's Festschrift, 1891, iii, p. 349.

5. Even with the best antagonism, both poisons are producing their action; as shown, for instance, by the greater liability of the protoplasm to depression.

6. Stimulation can be readily and completely removed by very small doses of a depressant (even by doses which are inactive when used alone).

7. Depression is lessened by only a limited range of doses of the stimulant. Its removal is in no case complete.

8. This gives to stimulation a limited but distinct usefulness.

9. The optimum dose of the stimulant is smaller when the embryos are depressed than when they are normal. Larger doses are synergistic to the depressant, and hence harmful.

10. The dose of the stimulant must therefore be chosen smaller, when depression exists, than in normal specimens.

I am indebted to Professor A. P. Mathews for much valuable advice in this research.

THE EFFECT OF DIURETICS ON THE URINE, WITH A DIET POOR IN SALTS.

By H. D. HASKINS.

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SOLLMANN has pointed out[1] that the administration of diuretics causes an increase of the urinary chlorides in rabbits whose urine has been rendered poor in these salts; but that they have no such effect in dogs.

He assumes, in explanation,[2] that the diuretics break down the resistance of the kidneys to the excretion of chlorides in the case of the more readily injured kidneys of the rabbit, but not in the case of the more resistant kidneys of the dog.

In accordance with Dr. Sollmann's suggestion, I undertook to determine how the normal human kidney would act under the conditions described above. For this purpose, the author, a young man in good health, was placed on a fairly uniform diet, poor in salts; when the urine had become fairly uniform, he took large doses of two typical diuretics belonging to different classes, namely, theobromin-sodium-salicylate (diuretin), and sodium acetate. Table I gives the data of the experiment. Three grams of diuretin failed to cause diuresis, the amount of chlorides, urea, and total solids in the twenty-four hours urine being less than on any preceding day. The diet was then made more liberal, because of a craving for solid food. This caused the chloride excretion to rise slightly. Nevertheless, twenty grams of sodium acetate failed to increase the amount of chloride excreted, although it did cause diuresis in so far as the water and urea excretion were concerned.

If the experiment had been continued longer, the chloride excretion would undoubtedly have fallen lower still; the chloride starvation was carried far enough, however, to warrant the conclusions drawn.

The normal human kidney, therefore, belongs to the resistant class.

[1] SOLLMAN, T.: This journal, 1903, ix, p. 446.
[2] SOLLMAN, T.: *Ibid.*, p. 451.

Day.	Diet.			Quantity of urine.	Specific gravity at 15° C.	Chlorides estimated as NaCl.		Urea.[1]		Total solids.[2]		Remarks.
	Total amt. of fluid.	Milk.	Chloride as NaCl.			Grams per litre.	Grams per day.	Grams per litre.	Grams per day.	Grams per litre.	Grams per day.	
	c.c.	grams.	grams	c.c.								
1st	2550	2040	5.517	980	1.0202	7.650	7.497	28.29	27.73	47.06	46.12	Weight, 134.75 lbs.
2d	2522	2267	6.217	1045	1.0203	4.699	4.911	32.79	34.27	47.30	49.43	
3d	2692	2267	6.217	800	1.0266	4.400	3.520	43.60	34.88	61.97	49.58	Perspired very freely.
4th	2607	2267	6.217	1240	1.016	2.048	2.540	26.29	32.61	38.28	46.23	
5th	2777	2267	6.217	1005	1.018	2.159	2.170	32.03	32.20	41.94	42.15	Diuretin, 1 gram at beginning of 24 hour period, 1 gram 3 hours later, 1 gram 6 hours later still.
6th	1870	(see remarks) 1360		1092	1.0183	2.490	2.720	25.00	27.30	39 04	42.64	Weight, 131.75 lbs. In addition to milk, took few ounces of stewed chicken, and 2 slices of bread and butter.
7th	2465	(see remarks) 1360	4.257	1050	1.0197	3.149	3.307	26.80	28.14	45.89	48.19	Also ate 4 eggs,[3] a few grams of peanuts[4] and 1 pear.
8th	2947	(see remarks) 2267	6.745	1415	1.0183	2.299	3.254	24.59	34.80	42.63	60.33	Ate same amount of eggs and peanuts as day before. Sodium acetate 10 grams, 3 hours after beginning of 24 hour period, 10 grams, 2 hours later.

[1] Urea was determined by the hypobromite method, the gas formed forcing water out of an intermediate bottle into a burette, barometric pressure being overcome by raising the bottle to get water in bottle and burette on same level before making the reading of displacement.

[2] Estimated from the specific gravity.

[3] The 4 eggs were found to contain chloride equivalent to 0.508 grams NaCl.

[4] Peanut meal was found to contain 0.4% NaCl.

SOME PHENOMENA OF ANIMAL PIGMENTATION.

By R. C. SCHIEDT.

WHILE engaged in a series of histogenetic investigations on the oyster, chiefly Ostrea virginiana and Ostrea edulis, carried on some ten years ago at Sea Isle City, Woods Hole, and Lancaster, Pa., I observed from time to time marked phenomena of pigmentation, which became so interesting to me that I concluded to make a special study of them. Preliminary notes of my results were first reported and published for me by the late Prof. Dr. John A. Ryder, in 1893, in the "Proceedings of the Academy of Natural Sciences" of Philadelphia, in the "Bulletin of the University of Pennsylvania," and in the "Annals and Magazine of Natural History of London." Since then I have further prosecuted my studies on this subject, and, although much has been written of late along the same line, I believe my conclusions present a new aspect worthy of consideration.

In order to obtain a perfect view of the function of the mantle edge of Ostrea virginiana, I placed a number of specimens, with the right valve removed, upon a wire netting stretched over one of the large tanks at the Sea Isle City Laboratory of the University of Pennsylvania, into which a constant stream of sea-water was flowing, covering the oysters several inches. The tank stood then inside of the Marine Laboratory at Sea Isle City, N. J., exposed to diffused sunlight. After a lapse of five days, I noticed that the oysters were covered by a brownish pigment, which in a few days grew almost black. The pigment spread over the whole of the epidermis of the exposed right mantle, the gills, heart, muscle, and body wall. At the same time the animals so exposed began to reproduce the lost valve and hinge, and even the insertion of the very small pedal muscle upon the inner face of the imperfectly reproduced right valve. The reproduction commenced at the hinge, so that in the mean time the right mantle was rolled up at the edge for lack of support, exposing the gills and the body wall (as seen in Fig. 1). The tank was removed to the outside of the laboratory and exposed to the direct sunlight. Most

of the oysters continued to live for fully four weeks. Although the adductor muscle was soon attacked by bacteria, and destroyed by putrefaction, the great splanchnic ganglion underlying it remained intact; the heart, although completely exposed through the tearing

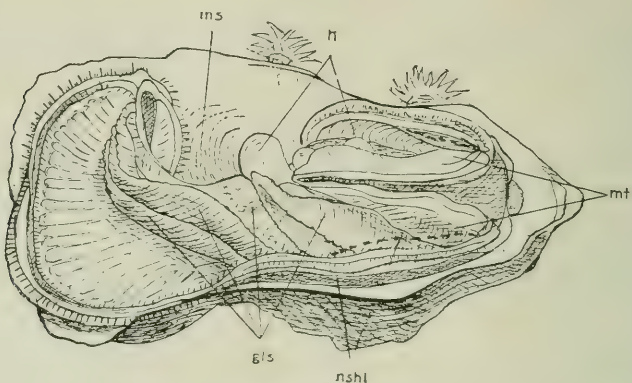

FIGURE 1. — Oyster, after ten days of exposure to direct light. *gls* = gills; *mtl* = mantle; *h* = heart; *nshl* = new shell; *ms* = muscle.

of the pericardium, continued to beat and propel the blood through the other organs of the body at the maximum rate of seventy-two pulsations per minute. Lack of time forbade the continuation of these experiments at Sea Isle City; but in the summer of 1894 I obtained

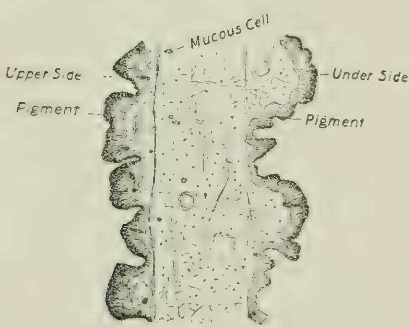

FIGURE 2.

FIGURE 3.

permission from United States Commissioner MacDonald to repeat the experiments at the United States Fish Commission Station at Woods Hole, Mass. The results hitherto reached had led me to the conclusion that light was the cause of pigmentation, and, in order to prove this more conclusively, I proceeded with a systematic investigation,

suggested by the composition and physical properties of light as exhibited by the numerous experiments in vegetable physiology.

I concluded that if pigmentation is the result of light effects, it could be best proved by exposing the animals to an-heliotropic as well as heliotropic rays, in order to show the difference. I, therefore, secured from the physiological laboratory of the Marine Biological School, through Prof. Dr. Jaques Loeb, then of Chicago University, who took a kindly interest in my experiments, pieces of dark blue and dark red glass, and arranged sets of oysters with the right valve removed in various tanks, exposing some to the direct rays of the sun, others only to red rays, a third lot only to blue rays, and a fourth lot I placed altogether in the dark. The dark red glasses transmitted only monochromatic light, while the dark blue transmitted a minute trace of red. One lot of oysters was exposed to direct sunlight from the 15th to the 25th of July; a second lot, from the 10th to the 28th of July; a third, from the 21st of July to the 5th of August; a fourth, not placed on wire but on sand, from the 21st of July to the 8th of August; a fifth, from the 21st of July to the 10th of August; a sixth, from the 21st of July to the 3d of August, and then taken back to the dark and kept there till the 16th of August. One of the oysters exposed to direct sunlight on the 1st of July survived till the 26th of July; another one lived from the 6th of July till the 1st of August. They were all placed along the same line, so that the light would strike them at the same angle. In all cases but one the muscles decayed; this one was exposed on the 10th of July, and still lived in a perfectly healthy and vigorous condition, without an apparent trace of a decayed muscle, on the 28th of July. The rest died, generally at the end of that length of exposure. In all cases a yellowish cast began to appear in a very few days, followed later by a brown one, and eventually by a still

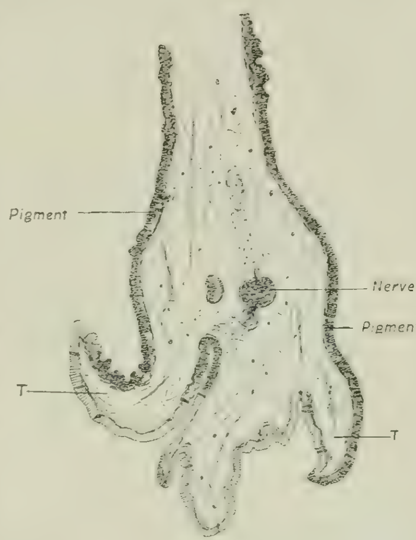

FIGURE 4

darker tint, until an almost black appearance was developed over the thus abnormally exposed parts of the mantle, gills, heart, and other organs, which are rarely or never pigmented in this animal. This pigment was largely diffused. It was mainly the uppermost surface of the organs that was abnormally exposed to the light that showed the effect of developing pigment. Thus, for instance, in the constriction between the auricles and the ventricles, the contraction of the pulsating heart would bring these parts together so frequently (seventy-two times per minute) as to shade the constriction between them, and here pigmentation was thus seemingly prevented. The undermost or shaded surfaces of the four gill pouches also remained much paler than the uppermost and exposed surfaces. This was, also, generally the case with other parts. Over the entire area thus abnormally exposed to the sunlight, there was developed, in the course of from fourteen to twenty days, a very pronounced pigmentation in excess of the normal; so marked, indeed, was this that in a number of instances the whole of the upper surface of the right mantle — the one usually exposed — became positively black. A number of oysters exposed to direct sunlight on the 21st of July were placed in the dark on the 3d of August, and were found to be greatly depigmented on the 16th of August.

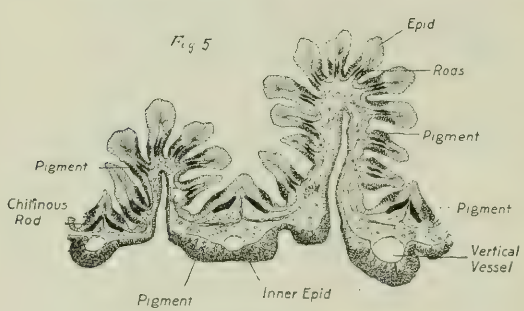

FIGURE 5.

Now as to the effect of blue light. One lot of oysters was placed under dark blue glass on a wire netting, also five or six inches below the surface of the water, from the 10th to the 25th of July; a second, from the 15th to the 31st of July; a third, from the 15th to the 25th of July; a fourth, from the 31st of July to the 10th of August. One of the second lot was after ten days placed in a small aquarium upstairs under the spout, and began to revive and look very healthy. In all cases the process of pigmentation was exactly the same as in those exposed to direct sunlight.

The effect of the dark red rays was entirely different. One lot of oysters was placed under the dark red glass from the 16th to the

31st of July; a second lot, from the 31st of July to the 8th of August. In no case did I discover signs of pigmentation; on the other hand, I found that the organs remained intact, and that the mantle was rapidly regenerated, which I attributed to the fact that the epidermis was protected against over-irritation and enabled to perform its normal work under favorable conditions.

Two lots were placed in darkness, one from the 15th to the 31st of July, and another from the 31st of July to the 10th of August. They all depigmented. One oyster, placed in the dark on the 10th of July, depigmented completely, and still lived on the 8th of August. There is very little pigment normally present in the oyster; it is most generally found in the epidermis of the mantle edge, but is very scattered. In the case of the oysters kept in the dark, and in perfectly clean tanks absolutely free from mud or even sand, the mantle edge became, in course of time, almost white.

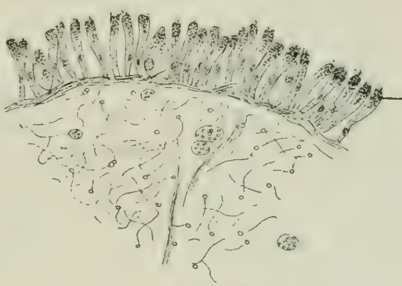

FIGURE 6.

To prove that I had really to deal with pigmentation, I made, in all cases, a series of sections through the mantle and the gill. The specimens (shown) were fixed either in one-half per cent of chromic acid, or in Flemming's Solution, then carried through the alcohols and embedded in paraffin. For the purpose of getting a clear idea of the cell structure, I always embedded one stained in borax-carmine or hæmatoxylin, and one unstained. On microscopic examination I found, to my surprise, that the pigmentation was confined to the epidermis, both in the mantle and in the gills. In the heart, of course, it was found embedded in the connective tissue. Figs. 2 and 3 represent transverse vertical sections through the mantle of oysters exposed to direct sunlight; the former had been exposed ten days, the latter eighteen days.

There is a decided difference in the density of the pigmentation of the two, No. 2 showing the pigment granules only on the outer surface of the epidermis, while in No. 3 the whole epidermis is densely pigmented, exhibiting special pigment cells. There is, likewise, seen a difference in the pigmentation between the upper and

lower surface in each case; but in both cases the pigment is confined to the epidermis. Fig. 4 shows a section through the mantle edge of an oyster that has been exposed to blue light for sixteen days. The result is the same, only that here the tentacles are not pigmented, because the mantle edge was turned over and the tentacles were shaded. Fig. 5 is a section through the gill of the same specimen. The lower epidermis is strongly pigmented, the upper only partly, owing to the fact that the gills also were turned up, but in neither case had the pigment reached the mesoderm. Fig. 6 is a section through the mantle hood of an oyster that had been exposed to diffused sunlight for ten days. The large goblet and glandular cells are distinctly pigmented at the outer edge. Fig. 7 represents the section through the gill of an oyster exposed for sixteen days to red light; it shows no pigmentation whatever. Fig. 8 is a transverse section through the mantle of the same specimen, showing the growth of the new shell, but no pigmentation.

FIGURE 7.

The results of these experiments agree with those described by Dr. Theo. List in Roux's Archiv für Entwicklungs Mechanik, 1899, viii, pp. 618–632. List's experiments were made on Mytilus and Lithodomus, but under the influence of direct sunlight only. He emphasizes the fact that pigmentation after cutting or laceration is abnormal. However, the heliotropic experiments with normal specimens of Eudendrium ramosum, made by Prof. Jaques Loeb, and described in Pflüger's Archiv für die gesammte Physiologie, 1896, lxiii, pp. 284–285, are an indorsement of my own results as far as the differences between the stimulating effects of red and blue light are concerned. Victor Faussek, on the other hand, failed to procure pigmentation in Mytilus. He says, in the "Zeitschrift für wissenschaftliche Zoologie," 1898, lxv, pp. 112–142: "In these experiments, as in those performed on oysters, I saw no trace of an effect of light upon the pigmentation of the animal. Exposure to bright light during many weeks produced no increased deposition of pigment, and many weeks in the dark caused no diminution in pigment. Under the action of light, there was neither an increased

pigmentation of normally pigmented surfaces, nor pigmentation in those parts, for example, the mantle, which had bent over and were recovering."

My experiments, repeated off and on for a period of ten years, at different latitudes, and in sea-water of different degrees of density, gave invariably the same results, so that I have not the slightest doubt as to their correctness.

These results are: —

1. Oysters deprived of one of their shells, and exposed to pure light, secrete pigment over the whole of their body.

2. The same pigment is also formed under the influence of the chemical or blue rays of light.

3. The heat rays or red rays of light are not favorable towards the formation of pigment, but also proof against pathological changes.

4. This pigment is in the ectodermal organs formed only in the epidermis.

5. In the organs of the mesoderm, *e. g.*, the heart, it is embodied within the endothelium and connective tissue fibres.

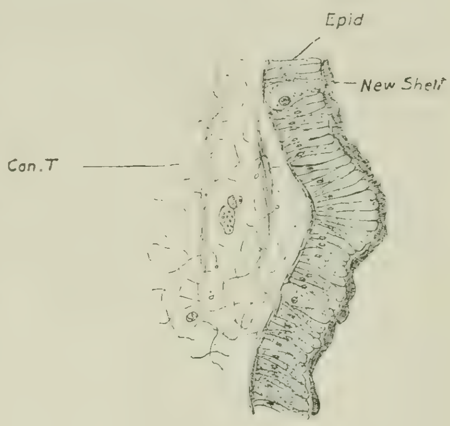

FIGURE 8.

6. All pigment disappears when the animal is placed in darkness.

Statement 4 differs materially from all other results hitherto obtained, especially in the vertebrate animals. Kölliker, Kerbert, Aeby, Riehl, and others maintain that all the pigment cells of the epidermis originate in the cutis, as pigmented migratory cells between connective tissue cells, whence they migrate into the epidermis; while Ehrmann thinks that the pigment cells of the cutis merely secrete pigment for the epidermis.

My experience leads me to maintain that the pigment in the ectodermal organ of the oyster is formed entirely in the epidermis, because it begins at the extreme outside margins, and moves towards the base of the ectodermal cells.

Moreover, Verworn, in his "Allgemeine Physiologie," Jena, 1897, says: "Almost the only secretions which continue in the organism

after their production are the pigments and the substances that form the skeleton." My experiments show that pigments produced under pathological conditions are not permanent. Also Kölliker, in his "Handbuch der Gewebelehre des Menschen," p. 203, says: "Kerbert found that in certain cases the pigment cells which wander into the epidermis, or are produced there, disappear."

I am inclined to think that pigment granules, as pointed out by Loeb, are excretory products of protoplasm caused by pathogenetic conditions, being re-absorbed again when the conditions are removed. The absence of the protective shell causes special irritation, perhaps a secretion of chromatin by the nucleus, and a restitution in darkness in which the animal is accustomed to live. This secretion of chromatin is closely connected with a chemical process, as shown by the effect of the chemical or blue rays. It can take place either in the ectodermal or in the mesodermal layer, the phenomenon not depending upon a specified tissue, but upon external conditions. Galeotti assumes that pigment granules are formed in the shell by special activity, particularly in the epithelium, where they have a colorless beginning, perhaps an excretion product of the nucleus.

FURTHER EXPERIMENTS ON THE INFLUENCE OF VARIOUS ELECTROLYTES ON THE TONE OF SKELETAL MUSCLES.

By W. D. ZOETHOUT.

IN a recent paper[1] on the effect of various salts on the tonicity of skeletal muscles it was shown that potassium, ammonium, cæsium, and rubidium chloride cause an increase in the tone of skeletal muscle, and that calcium, strontium, and magnesium chloride, and, to a lesser extent, sodium and lithium chloride, are able to counteract the effects of the first-mentioned salts. This work, which was continued during the summer of 1902 at the University of Chicago, under the direction of Dr. Loeb, showed that alkalies also increase the tone of striated muscles, and that this action is reversed by acids and various salt solutions.

When the gastrocnemius muscle of a frog is placed in $\frac{m}{8}$ sodium hydrate, the tone of the muscle is immediately increased. This is also true for the hydrates of potassium, ammonium, barium, and strontium. In such solutions the irritability of the muscle is speedily destroyed; hence in all these experiments more dilute solutions were used, the hydrate being in all cases diluted with $\frac{m}{8}$ sodium chloride solution, unless otherwise stated. The minimum concentration of sodium hydrate which can call forth an increase in the tone is $\frac{m}{400}$; weaker solutions may occasionally cause a very slight shortening of the muscle.

When a muscle is first treated with $\frac{m}{80}$ sodium hydrate, and subsequently with $\frac{m}{80}$ hydrochloric acid, the increase of tone induced by the hydrate is reversed by the acid. But acids, like alkalies, increase the tone. If, therefore, in the above experiment, the acid is allowed to act for a sufficient length of time, the decrease in tone is followed by a second increase. If now the acid is again replaced by the alkali, the tone is at first again diminished, to be increased after a short latent period. This process can be repeated four or five times,

[1] ZOETHOUT: This journal, 1902, vii, p. 199; 1904, x, p. 211.

FIGURE 1.—At X the muscle is placed in 1 c.c. $\frac{m}{8}$ NaOH + 9 c.c. $\frac{m}{8}$ NaCl. At A this is exchanged for $\frac{m}{8}$ NaCl; at B for $\frac{m}{80}$ NaOH; at C for $\frac{m}{80}$ HCl; at D for $\frac{m}{80}$ NaOH; at E for $\frac{m}{80}$ HCl.

provided the acid and alkali solutions are not too concentrated and are not allowed to remain in contact with the muscle for too long a time (see Fig. 1).

The power to reverse the action of alkalies is also shared by certain salt solutions. At X, in Fig. 1, the muscle was treated with $\frac{m}{80}$ sodium hydrate; the tone was almost immediately increased. After one minute (at A) the alkali was replaced by $\frac{m}{8}$ sodium chloride solution, and the muscle regained its normal length in about five minutes. This experiment was also made with the hydrate of potassium, barium, and strontium, and the same results obtained. The increase of tone induced by alkalies can also be reversed by lithium chloride solutions. If the concentration of the sodium hydrate is greater than $\frac{m}{80}$, the sodium or lithium chloride solutions still tend to abolish the increase of tone, but the reversal is never so complete as when weaker alkalies are employed.

The increase of tone induced by hydrates having a concentration of $\frac{m}{100}$ or less is also reversed by the subsequent application of calcium chloride and strontium chloride and iodide. But when the concentration of the hydrate is greater than $\frac{m}{100}$, the subsequent treatment with calcium or strontium chloride does not diminish the tone, but increases it still more. This is shown in Figs. 2 and 3. In Fig. 2 the muscle was first treated with $\frac{m}{200}$ sodium hydrate solution, which produced an increase in tone. After two minutes (at B) the alkali was replaced by $\frac{m}{8}$ calcium chloride, and the tone was immediately decreased. Fig. 3 was obtained in the same way, except that $\frac{m}{52}$ instead of $\frac{m}{200}$ sodium hydrate was employed. The subsequent application of calcium chloride (at B) did not cause a relaxation, but a further increase of tone. The same results were obtained with strontium chloride and iodide. The increase in tone caused by calcium chloride, when this replaces a strong sodium hydrate solution, is reversible by sodium or lithium chloride solutions.

When $\frac{m}{100}$ sodium hydrate solution is replaced by barium nitrate or chloride, the increase of tone in-

duced by the alkali is to some extent reversed, but great rhythmical contractions appear.[1] This peculiar action of calcium and strontium is also seen in the following experiment. The left gastrocnemius of a frog was placed in a mixture of 0.5 c.c. $\frac{m}{8}$ NaOH + 9.5 c.c. $\frac{m}{8}$ NaCl.

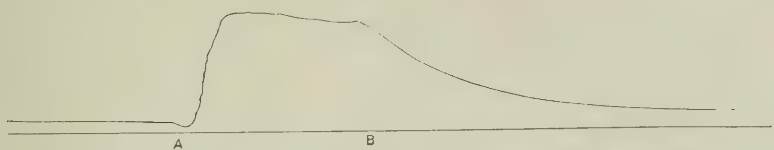

FIGURE 2. — At *A* the muscle is placed in 0.4 c.c. $\frac{m}{8}$ NaOH + 9.6 c.c. $\frac{m}{8}$ NaCl. At *B* this is replaced by $\frac{m}{8}$ $CaCl_2$.

The tone was immediately increased (Fig. 4), and this increase was maintained throughout the entire length of the experiment — thirty-five minutes. The right gastrocnemius muscle of the same frog was placed in a mixture of 0.5 c.c. $\frac{m}{8}$ NaOH + 9.5 c.c. $\frac{m}{8}$ $CaCl_2$. At first a slight relaxation took place (Fig. 5), which is followed, after a latent period of about eight minutes, by a very gradual increase in tone, but at the end of the thirty-five minutes the increase of tone in this muscle is almost twice as great as in the other muscle.

This difference between the sodium and the calcium salts cannot be attributed to the bivalency of the calcium, for magnesium salts do not behave like the calcium salts. When a $\frac{m}{27}$ sodium hydrate solution is replaced by $\frac{m}{8}$ magnesium chloride, the action of the alkali is reversed. This difference between the action of calcium and magnesium is illustrated in Fig. 6. After the muscle had been immersed in the sodium hydrate for one-fourth minute, it was subjected to $\frac{m}{8}$ magnesium chloride (at *a*, Fig. 6) and a relaxation followed. After twenty-six minutes the magnesium chloride was replaced by calcium chloride (at *b*) which caused the muscle to shorten. The difference between the salts of these two bivalent metals might be attributed to the fact that magnesium chloride precipitates the hydrate more

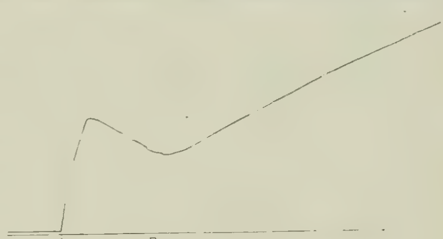

FIGURE 3. — At *A* the muscle is placed in 1.5 c.c. $\frac{m}{8}$ NaOH + $S\frac{1}{2}$ c.c. $\frac{m}{8}$ NaCl. At *B* this is replaced by $\frac{m}{8}$ $CaCl_2$.

[1] *Cf.* LOEB: The Decennial Publications of the University of Chicago, 1902, x, p. 4.

completely than does the calcium or strontium chloride. This, however, is not the correct explanation, for while the sodium salts do not

FIGURE 4. — At *A* the muscle is placed in 0.5 c.c. $\frac{m}{8}$ NaOH + 9.5 c.c. $\frac{m}{8}$ NaCl.

precipitate the hydrate at all, yet these salts act like the magnesium and not like the calcium or strontium salts.

While making the above experiments with the hydrates, it occurred to me to test the action of the carbonate of sodium, potassium, and lithium; experiments showed that these carbonates act much like the hydrates. Of the three salts the potassium carbonate causes the

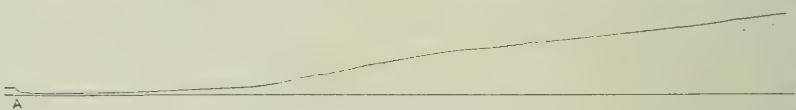

FIGURE 5. — At *A* the muscle is placed in 0.5 c.c. $\frac{m}{8}$ NaOH + 9.5 c.c. $\frac{m}{8}$ $CaCl_2$.

greatest and the lithium carbonate the smallest increase in tone. This is in accord with former results.[1] Potassium chloride increases the tonicity, sodium chloride counteracts this effect, and lithium chloride does so to a still greater extent. Hence, it was to be expected that the potassium carbonate would have the greatest effect and the lithium carbonate the least. The potassium and lithium

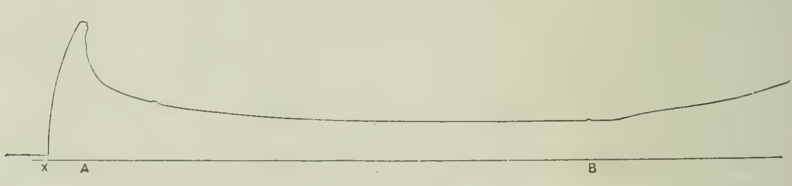

FIGURE 6. — At *X* the muscle is placed in 3 c.c. $\frac{m}{8}$ NaOH + 7 c.c. $\frac{m}{8}$ NaCl. At *A* this is replaced by $\frac{m}{8}$ $MgCl_2$, and at *B* this is replaced by $\frac{m}{8}$ $CaCl_2$.

carbonate also have little or no tendency to cause rhythmical contractions of the muscle, a characteristic so prominent in the carbonate of sodium.

The minimum concentration of sodium carbonate necessary to cause a very slight increase in the tonicity is a $\frac{m}{27}$ solution. The

[1] ZOETHOUT: This journal, 1904, x, p. 211.

increase of tone thus produced is reversible by sodium and lithium chloride solutions.

As it is highly probable that the power of the carbonates to increase the tone is due to the OH-ions formed in the solution, the calcium and strontium salts ought to act here as they did in the case of the hydrate. When a muscle is placed for two or three minutes in $\frac{m}{8}$ sodium carbonate, the subsequent application of calcium or strontium chloride produces an immediate relaxation. But if the muscle be left in $\frac{m}{8}$ solution of any of the carbonates for twenty or thirty minutes, the subsequent treatment with calcium or strontium chloride does not reverse the increase of tone caused by the carbonates, but produces a still further shortening of the muscle.

Summary.

1. The hydrates of sodium, potassium, ammonium, barium, and strontium cause an increase in the tone of skeletal muscle.

2. This increase in tone is abolished by sodium, lithium, and magnesium chloride solutions.

3. If the increase in tone is produced by a weak solution of the hydrate ($\frac{m}{100}$) the salts of calcium, strontium, and barium also reverse the action. If the concentration of the hydrate is greater than $\frac{m}{100}$, the calcium, barium, and strontium salts cause a further shortening of the muscle.

4. The carbonates of sodium, potassium, and lithium are very similar to the hydrates in their action on the tone of skeletal muscle.

EFFECTS OF CERTAIN SALTS ON KIDNEY EXCRETION, WITH SPECIAL REFERENCE TO GLYCOSURIA.[1]

By ORVILLE HARRY BROWN.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

A TRANSIENT glycosuria occurs under a great number of conditions and is an accompanying symptom of more or less importance in a great variety of maladies. Experimentally it is well known that a transient increase of sugar in the urine can be produced in a variety of ways, chief of which are the use of phlorhizdin, adrenalin chloride, excessive amounts of narcotics, extirpation of the pancreas, a puncture of the fourth ventricle, and by large amounts of sugar taken into the alimentary canal. In marked diuresis sugar is usually found in the urine. Jacobj[2] showed that in rabbits glycosuria always accompanied the diuresis, produced by caffein, caffein sulphate, and theobromine. Bock and Hoffman[3] found sugar in the urine after intravenous injections of large quantities of a 1 per cent solution of sodium chloride. Kessler[4] observed that sugar was constantly in the urine of cats after injections of sodium carbonate.

At the suggestion of Dr. A. P. Mathews, I undertook a series of experiments to ascertain the effect of salt solutions on the production of diuresis, and on the excretion of sugar by the kidneys. If the kidney activities are influenced by ions as are the functions of other

[1] Since this paper has been sent to the Journal, reprints from Dr. MARTIN H. FISCHER, of a preliminary report in the University of California Publications, on "The Production and Suppression of Glycosuria in Rabbits through Electrolytes," have been received. It is certainly gratifying that his results, as far as reported, corroborate mine. I wish to say that practically all of my results were obtained during the winter quarter of 1903, and have been publicly announced several times by Dr. A. P. MATHEWS. See Yale Medical Journal, June, 1903.

[2] JACOBJ: Archiv für experimentelle Pathologie und Pharmakologie, 1895, xxxv, p. 213.

[3] BOCK and HOFFMAN: Archiv für Anatomie und Physiologie, 1871, i, s. 550; JACOBJ: *Loc. cit.*

[4] KESSLER: Versuch uber die Wirkung einiger Diuretics, Dissertation, 1877, Dorpat; JACOBJ: *Loc. cit.*

tissues of the body, then we have reason to assume that the renal excretion, either in quality or quantity, or both, would be increased by some ions and decreased by others. Dr. A. P. Mathews [1] has shown that nerve fibres are stimulated by anions and depressed by cations. Loeb [2] has shown that the nature of the ions in a solution in which a muscle is immersed has an important influence on the muscle contraction. Cole,[3] and Neilson and Brown [4] have demonstrated that the activities of enzymes and catalytic agents are retarded by cations and accelerated by anions.

Methods.

Rabbits were employed for the experiments. A 6 per cent solution of urethane given by the stomach was used as the anesthetic, $1\frac{1}{2}$ gm. being given per kilo weight. The solutions used were injected into the jugular vein. The urine was collected from a cannula which was inserted into the bladder. The urine was tested for sugar, qualitatively by Haines's [5] solution, fermentation, and the polarimeter, and quantitatively by either Fehling's solution or the polarimeter, or both. As there was always a possibility of sugar's being produced in the urine, by the operation combined with the anesthetic, the solutions were not injected until it had been shown that there was no glycosuria, or in case there was some, until the urine no longer gave a test for sugar. There were but a few experiments where sugar appeared after the operation before the injection of a solution. If it appeared, it usually disappeared within one or two hours.

The experiments given below were repeated a number of times with similar results. In some of the phlorhizdin experiments calcium chloride was used in place of the strontium chloride. It had practically the same effect, but possibly had a greater tendency to decrease the amount of water excreted. There is, however, a depression of water excretion by both the calcium and strontium chlorides when they are used in sufficient quantities. In a number of cases complete or nearly complete anuria was produced by suddenly increasing the

[1] Mathews: Science, n. s., 1903, No. 436, p. 729.

[2] Loeb: This journal, 1900, iii, p. 383.

[3] Cole: Journal of physiology, 1903, xxx, p. 281.

[4] Neilson and Brown: This journal, 1904, x, p. 225.

[5] Haines's solution is the same as Fehling's solution except that the copper sulphate and alkali are kept together, with sufficient glycerine added to dissolve the copper hydrate.

amount of calcium or strontium chloride injected. This tendency toward suppression of the kidney excretion was much more marked with one lot of rabbits which was used than with the rest. The explanation for this I do not know. Dr. S. A. Matthews[1] has observed that anuria can be produced by the use of an $\frac{m}{8}$ solution of gold chloride. In smaller amounts he has found that the gold chloride inhibits the excretion of sugar produced by those salts which produce diuresis. It was also observed during the course of my experiments that sodium acetate, sodium citrate, and barium chloride caused a very active peristalsis which in some cases resulted in the defecation of a large amount of watery fæces. It should be observed that sodium chloride and sodium citrate produce a reducing substance in the urine, even though the amount of urine excreted was in some cases very much less than the amount of the solution injected. This substance in the first few experiments was tested only by the use of Haines's solution. The reduction, however, resembled closely that resulting from a known sugar, placed in sugar-free urine.

Experiment 1. — Rabbit, weight, 2500 gms. 10 c.c. urine in 45 minutes. No sugar. 20 c.c. $\frac{m}{8}$ sodium acetate injected slowly. 40 c.c. of urine excreted. This gave a heavy reduction with Haines's solution.

Experiment 2. — Rabbit, weight, 2100 gms. 8.45 to 9.30 A.M., 14 c.c. of urine were collected. No sugar. At 9.30 a slow injection of sodium citrate was begun. Amount excreted was practically equal to amount injected. This urine gave a heavy reduction with Haines's solution.

Experiment 3. — Rabbit, weight, 1880 gms. From 2.00 to 3.00 P.M., 21 c.c. of urine were excreted. No sugar. From 3.00 to 3.20, 16 c.c. of an $\frac{m}{8}$ sodium sulphate were injected. At 4.30, $1\frac{1}{2}$ hours after the beginning of the injection, 43 c.c. of the solution had been injected, and 185 c.c. of urine had been excreted. This urine gave a heavy reduction with Haines's solution.

Experiment 4. — Rabbit, weight, 1700 gms. From 9.00 to 10.00 A.M., 10 c.c. of urine were collected. No glycosuria. From 10.00 to 12.00, 20 c.c. of $\frac{m}{8}$ sodium chloride were injected. 40 c.c. of urine collected. Heavy reduction of Haines's solution was produced by this urine.

Experiment 5. — Rabbit, weight, 1900 gms. The urine following the operation gave a marked reduction of Haines's solution. After $1\frac{1}{2}$ hours this had disappeared. During the next hour a mixture of 50 c.c. $\frac{m}{8}$ sodium

[1] Personally communicated.

chloride, 25 c.c. $\frac{m}{8}$ sodium sulphate, and 25 c.c. $\frac{m}{8}$ sodium citrate, was injected. 87 c.c. of urine were collected, and sugar was shown by Haines's solution and fermentation to be present in considerable quantities.

Experiment 6. — Rabbit, weight, 1700 gms. Reducing substance in urine, after the operation. From 10.00 to 12.00 injected 100 c.c. of an $\frac{m}{8}$ solution of sodium chloride. This urine produced considerable gas on fermentation, and gave a heavy reduction with Haines's solution. From 12.00 to 1.30, $97\frac{1}{2}$ c.c. of $\frac{m}{8}$ sodium chloride and $2\frac{1}{2}$ c.c. of $\frac{m}{8}$ calcium chloride were injected. $27\frac{1}{2}$ c.c. of urine were collected, and on fermentation only a small amount of gas was given off, and on treating with Haines's solution only a slight precipitate was produced. From 1.30 to 3.00, 95 c.c. of sodium chloride and 5 c.c. of $\frac{m}{8}$ calcium chloride were injected. 50 c.c. of urine were excreted. Neither fermentation nor reduction of Haines's solution took place.

Experiment 7. — Rabbit, weight, 1500 gms. 8 c.c. of urine from 10.00 to 11.00. No sugar. This experiment was the same as 6, except strontium chloride was substituted for calcium chloride. The sugar which was produced by the sodium chloride was prevented by the addition of small amounts of strontium chloride to the sodium chloride. The specific gravity of urine was 1.016 during the sodium chloride injection, and 1.005 after the addition of the strontium chloride. From 3.00 to 5.00, injected 200 c.c. of a mixture[1] of sodium chloride, sulphate, and citrate, in the proportion of 50 c.c. of an $\frac{m}{8}$ solution of the first, 25 c.c. of an $\frac{m}{4}$ solution of the second, and 25 c.c. of an $\frac{m}{12}$ solution of the third. 275 c.c. of urine were removed. 0.005 gm. of sugar per c.c. of urine were shown by Fehling's solution to be present. From 5.00 to 7.00 P.M., injected 200 c.c. of a solution the same as the one mentioned just preceding, except that in 100 c.c. of the mixture, there were 45 c.c. of sodium chloride instead of 50 c.c., $22\frac{1}{2}$ c.c. of sodium citrate instead of 25, and $7\frac{1}{2}$ c.c. of $\frac{m}{8}$ strontium chloride was added. 130 c.c. of urine were excreted. By the same test as above, 0.002 gm. of sugar per c.c. of urine were shown to be present. The last few c.c. of urine collected gave no sugar test.

Experiment 8. — Rabbit, weight, 1710 gms. No sugar in urine following the operation. Injected slowly a mixture of 50 c.c. of $\frac{m}{8}$ sodium chloride, 25 c.c. of $\frac{m}{4}$ sodium sulphate, 20 c.c. of $\frac{m}{12}$ sodium citrate, and 5 c.c. of $\frac{m}{8}$ calcium chloride. Test for sugar in urine excreted was negative.

[1] This, with a small amount of $\frac{m}{8}$ calcium chloride, is the combination used by Dr. S. A. Matthews for producing diuresis and successfully applied by him in the treatment of tetanus.

Experiment 9. — Rabbit, weight, 1330 gms. Given a subcutaneous injection of 5 c.c. of a 2 per cent alcoholic solution of phlorhizdin. Sugar was found in the urine after 20 hours.

Experiment 10. — Rabbit, weight, 1540 gms. No sugar an hour after operation. From 11.30 to 1.30, 80 c.c. of $\frac{m}{8}$ sodium chloride and 20 c.c. of $\frac{m}{8}$ strontium chloride were injected. At 12.00, 5 c.c. of phlorhizdin, the same solution as used above, was given subcutaneously. At 2.00, 50 c.c. of urine were excreted. No glycosuria. The flow of urine became very slow, and 50 c.c. of an $\frac{m}{8}$ sodium chloride were injected, and by 6.00 o'clock, 30 c.c. of urine had been excreted. Just a very slight reduction occurred when treated with Haines's solution.

Experiment 11. — Rabbit, weight, 1300 gms. No sugar in urine for an hour after operation. From 10.30 A.M. to 12.30 P.M., a mixture of a 95 c.c. of $\frac{m}{8}$ sodium chloride, and 5 c.c. strontium chloride, and 5 c.c. of the phlorhizdin as used before, was injected. Samples of urine collected up to 12.30 A. M. gave only in two or three cases slight traces of sugar.

Experiment 12. — Rabbit, weight, 1570 gms. At 9.15 there was a slight amount of reducing substance. None at 10.45. At 10.45, injected 25 c.c. of an $\frac{m}{8}$ solution of sodium chloride. At 12.30, 27 c.c. of urine were excreted. No sugar. At 12.30, injected 25 c.c. sodium chloride and $\frac{1}{2}$ c.c. phlorhizdin. Within about 15 minutes sugar was found in the urine. By 2.45, 30 c.c. of urine were excreted, the last samples of which contained no sugar. The urine, tested quantitatively by the polariscope, showed that 0.2 gm. of sugar were excreted. My readings of the polariscope were verified in every case by Dr. Guthrie. At 3.00 injected 24 c.c. of $\frac{m}{8}$ sodium chloride and $\frac{1}{2}$ c.c. of phlorhizdin, same as before used. By 5.20, 26 c.c. of urine had been excreted, the last of which contained no sugar. The amount of sugar excreted this time, tested in the same manner as before, was 0.05 gm.

Summary and Conclusions.

1. An $\frac{m}{8}$ solution of sodium chloride, an $\frac{m}{8}$ solution of sodium citrate, an $\frac{m}{8}$ solution of sodium acetate, and an $\frac{m}{8}$ solution of sodium sulphate, as observed by Dr. S. A. Matthews [1] and others, and corroborated by my experiments, produces diuresis.

2. These same salts, as has been observed by Mock and Hoffman [2] in case of sodium chloride, produce a glycosuria.

[1] Dr. S. A. Matthews: Personally communicated.

[2] Mock and Hoffman: *Loc. cit.*

3. It might seem that the diuresis was the cause of the glycosuria, except that by an addition of small amounts of calcium or strontium chloride, the glycosuria is prevented while the diuresis still exists.

4. Calcium and strontium chlorides likewise decrease or totally inhibit the excretion of sugar which is caused by the injection of phlorhizdin.

5. Since diuresis and glycosuria are caused by those salts which have been found by Dr. A. P. Mathews [1] to stimulate nerve, and that depression of these activities is caused by salts which he has found to depress the irritability of nerve, we may assume that the anions stimulate the renal activities, and the cations depress them.

6. It was not ascertained whether the glycosuria caused by the salts was the same as that produced by phlorhizdin, or whether the sugar of the blood was increased. Further experiments are being carried on in this laboratory to determine this, and to ascertain the practical value of results obtained.

I take this opportunity to express my thanks to the members of the laboratory, especially Dr. A. P. Mathews and Dr. S. A. Matthews.

[1] Dr. A. P. Mathews: *Loc. cit.*

ON THE MORPHOLOGICAL CHANGES IN THE BLOOD AFTER MUSCULAR EXERCISE.

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CONTENTS.

I. Historical.

COMPARATIVELY few investigations have been made upon the influence of muscular work upon changes in the blood.

Lloyd Jones,[1] in a series of experiments upon the variations in the specific gravity of the blood in health, studied among other things the influence of muscular exercise. This investigator mentions a fall in the specific gravity of the blood from 1.0605 to 1.059 following a walk of two miles on a cold day, whilst a run of four and one-half miles caused a rise in specific gravity 1.0585 to 1.061. On another occasion tennis-playing on a cool evening caused a fall in specific gravity from 1.061 to 1.0602, whereas the same form of exercise taken during a very warm afternoon caused the specific gravity of the blood to rise to 1.063. Jones concluded that muscular exercise, if gentle and not too prolonged, would cause a fall in the specific gravity of the blood, but if perspiration became well marked, or if more violent

[1] Jones: Journal of physiology, 1887, viii, p. 1.

exercise was indulged in, the specific gravity of the blood would be considerably increased. Schmaltz,[1] after exercising actively for ten minutes in a gymnasium, noticed a fall in the specific gravity of the blood from 1.0588 to 1.058. At the same time the specific gravity of the urine increased from 1.018 to 1.023.

The leucocytosis following muscular work has been investigated by Larrabee.[2] He examined the blood of four contestants in the Boston Athletic Association's Marathon Race of 1901. This was a road race twenty-five miles in length and the distance was travelled by the winner in about two and one-half hours. Five minutes after the finish a leucocytosis was found ranging from 14,400 to 22,200. The author's data indicate that prolonged physical exercise of a violent and exhausting nature produces a pronounced leucocytosis. Schulz[3] found a maximum leucocytosis of 13,600 following muscular work, while Burrows,[4] in a single experiment upon a healthy young athlete, found a leucocytosis of short duration varying between 11,400 and 17,000.

The change in the number of red corpuscles and of leucocytes under the influence of muscular exercise has been investigated by Willebrand.[5] This observer used twelve young men as subjects, and made fourteen experiments. The muscular exercise taken was of a gymnastic character and was generally continued for about ten minutes. One blood count was made before the exercise, and two after the exercise was completed, the first of these two counts being made within ten minutes and the other after the lapse of a period not exceeding one and one-half hours. In seven experiments in which the number of the red corpuscles was determined, the increase varied between 2.9 per cent and 23.4 per cent, and an average increase of 12.3 per cent was observed. The length of time consumed in returning to the normal was quite variable. In some experiments the normal number of red corpuscles was reached in one and one-half hours, while in other experiments the maximum number of corpuscles was found after the lapse of a period of similar length. The leucocytes became normal in number somewhat more quickly and in a much more uniform manner than the red corpuscles. The increase

[1] Schmaltz: Deutsches Archiv für klinische Medicin, 1890–91, xlvii, p. 145.

[2] Larrabee: Journal of medical research, new series, 1902, ii, p. 76.

[3] Schulz: Deutsches Archiv für klinische Medicin, 1893, li, p. 234.

[4] Burrows: American journal of the medical sciences, 1899, cxvii, p. 503.

[5] Willebrand: Skandinavisches Archiv für Physiologie, 1903, xiv, p. 176.

in leucocytes ranged from 19.2 per cent to 96.9 per cent, the average leucocytosis being 47 per cent. Cohnstein and Zuntz[1] performed the unique experiment of examining the blood of rabbits after subjecting the animals to a systematic harassing (hetzen) for several minutes. Their data show a slight decrease in the number of red corpuscles and an accompanying leucocytosis. Cadet[2] examined the blood of a few men in April and again in September after the lapse of five months passed in very severe muscular work. No change was noticed in the number of leucocytes, but the red corpuscles were decreased 1,100,000 in one case and at least 600,000 in each of four other cases.

During a series of twenty-eight marches made by a body of German troops extensive investigations were made upon the soldiers under the direction of Zuntz and Schumberg.[3] The marches varied from 18 to $24\frac{3}{4}$ kilometres, and the equipment carried by each man varied from 22 to 31 kilograms on the different marches. The examinations of the blood were made by Tornow.[4] He observed an increase in specific gravity of from 0.002 to 0.006, an average increase of 9 per cent in the number of red corpuscles and an average increase of 43 per cent in the number of leucocytes. Mitchell[5] reports an increase in the number of red corpuscles from 5,650,000 per cubic millimetre to 8,125,000 per cubic millimetre as the result of a massage for one hour. He examined over thirty cases and concludes that massage increases the number of red corpuscles, and to a lesser extent and not so constantly their hæmoglobin content. This same investigator reports a red corpuscle count of 6,800,000 in a man who had walked two and a half miles on a very cold windy day. No examination was made of the normal blood of this individual, but the number of red corpuscles was obviously considerably increased as a result of the muscular work incident to the long walk.

Observations upon the influence of cold baths have been made by

[1] Cohnstein and Zuntz: Archiv für die gesammte Physiologie, 1888, xlii, p. 303.

[2] Cadet: Étude physiologique des éléments figurés du sang, Paris, 1881. Quoted by Tornow (see above).

[3] Zuntz and Schumberg: Studien zu einer Physiologie des Märsches, Berlin, 1901. Quoted by Willebrand: *Loc. cit.*

[4] Tornow: Blutveränderungen durch Märsche, Inaugural-Dissertation, Berlin, 1895, pp. 1–66. Quoted by Willebrand: *Loc. cit.*, and by Krehl: Pathologische Physiologie, Leipzig, 1898, p. 158.

[5] Mitchell: American journal of the medical sciences, 1894, cvii, p. 502.

Winternitz[1] and by Thayer.[2] Winternitz noted an increase in the number of red corpuscles and leucocytes following cold baths, and further demonstrates that slight muscular movements without the application of cold cause a similar increase. Thayer concludes from his experiments that in most cases of prolonged cold baths a marked and rapidly appearing leucocytosis is noted. He was uncertain whether the leucocytosis was general or local. A marked increase in the number of leucocytes was noted within a half-hour after the beginning of a bath, and the most pronounced leucocytoses occurred when the bath was accompanied by intense shivering. Knöpfelmacher[3] investigated the influence of both cold and hot baths. After the former he observed a maximum increase of 30 per cent in the number of red corpuscles. After baths taken at a temperature of 38–42° C. a maximum decrease of 23 per cent in the number of red corpuscles was noted, while the variation in the number of the leucocytes was irregular. An increase in the number of red corpuscles was noted after very hot baths, and a relatively much greater increase in leucocytes. Tarchanoff[4] in several experiments found a considerable increase in the hæmoglobin value one half-hour after a Russian steam bath.

The influence upon the blood of the muscular activity accompanying convulsions has been studied by Capps,[5] Michea,[6] and by Burrows.[7] The first observer noted a rise in the number of red corpuscles and leucocytes at the occurrence of a convulsion. The leucocytosis varied quite regularly with the severity and duration of the seizure, and after very severe convulsions as many as 18,000 leucocytes per cubic millimetre of blood were found. Burrows made similar observations and claimed that part of the leucocytosis following a convulsion was due to the muscular work accompanying the seizure, and that the remainder of the leucocytosis was of a pathological nature and was of longer duration than the leucocytosis due to the muscular exertion. The author cites a leucocytosis of 16,600 followed by a fall to 6,200.

[1] Winternitz: Centralblatt für klinische Medicin, 1893, xiv, p. 177.
[2] Thayer: Johns Hopkins Hospital Bulletin, 1893, iv, p. 37.
[3] Knöpfelmacher: Wiener klinische Wochenschrift, 1893, vi, p. 810.
[4] Tarchanoff: Archiv für die gesammte Physiologie, 1881, xxiv, p. 525.
[5] Capps: American journal of the medical sciences, 1896, cxi, p. 650.
[6] Michea: Zeitschrift für Psychiatrie, 1848, v, p. 485.
[7] Burrows: *Loc. cit.*

II. Experimental.

1. Purpose of the experiments, subjects, etc. — The purpose of these experiments was to study the *immediate* variation in the number of red corpuscles and leucocytes in the blood of young, healthy college athletes following participation in the various forms of athletic activity peculiar to the preliminary training and actual competition in which the college athlete ordinarily engages. Several experiments were made upon athletes during actual competition in the annual Inter-class Track Meet of the University of Pennsylvania, while other tests were made upon the participants in the swimming contests held in the tank at Houston Hall. Still other experiments were made upon athletes holding friendly competitions during their preliminary training, while further experiments were made when the men were indulging in routine training and the stimulus of competition was absent. In all, fifty experiments were made upon twenty-two subjects, the periods of muscular exertion varying from a few seconds to about one hour. The experiments on the swimmers were made during the evening, and in general the other experiments were made during the afternoon.

The subjects were young men between seventeen and twenty-nine years of age, and, with a single exception, were students of the University of Pennsylvania. The greater number of these young men were of pronounced athletic tendencies, and through the medium of their daily exercise they were presumably in good physical condition. Several of the men were regular members either of the track team, lacrosse team, cross country team, or water polo team, while others were in active training with the object of securing a position on one of the university athletic teams.

2. Taking the blood sample, counting the blood cells, etc. — In taking the blood for examination the finger was punctured by means of a sterile needle, and the blood was allowed to flow freely from the puncture, no pressure being used.

Both the leucocytes and the red corpuscles were counted, use being made of the Thoma-Zeiss Hæmocytometer. By means of a Zappert[1] slide both the red corpuscles and the leucocytes were counted in the same specimen of blood. Toison's solution was the diluting fluid used.

[1] Zappert: Centralblatt für klinische Medicin, 1892, No. 19.

Whenever circumstances would permit, the blood of the subjects was examined before and after the exercise. Ordinarily a blood sample was taken in the dressing-room immediately preceding the exercise, and upon the return of the subject from the track, field, or swimming tank the second sample of blood was taken. It was aimed to take this second sample not later than two minutes after the exercise had ended. The first sample was always taken as near the beginning of the exercise as circumstances would permit, but there was frequently an interval of one half-hour between the taking of the blood sample and the commencement of the exercise. It is evident, however, that a few minutes' variation in the time of taking the blood for this preliminary counting would probably make no real difference, as the normal blood count would obviously not materially change in such a short period of time. With the count succeeding the exercise, however, conditions were different, as changes might possibly occur very rapidly. For this reason the sample of blood taken after exercise was secured immediately upon the return of the subject to the dressing-room. In a few instances where the circumstances were such as to make impossible the taking of a blood sample before exercise, the blood count was taken at the end of the exercise period, and the normal blood count was estimated. This second method is obviously less accurate than the first method, yet we feel that, as we were dealing with a single class of subjects, *i. e.*, young men in active athletic training, and that as the normal blood counts as determined varied within such narrow limits, the estimates, made only after carefully noting all the conditions, cannot fail to give a very close approximation to the actual blood count.

The author wishes to thank Prof. John Marshall and Prof. C. C. Stewart for valuable suggestions. His thanks are also due Messrs. Shell and Kistler for affording the author facilities in the dressing-rooms at Franklin Field and Houston Hall. To the following gentlemen who so kindly acted as subjects during the progress of these experiments, the author wishes to express his gratitude: E. S. Amsler, A. E. Carpenter, E. Cowlishaw, L. M. Craver, B. F. Erwin, F. R. Forster, J. C. Gilpin, "Mr. Goulder," A. Goldwater, H. A. Hyman, R. H. Ivy, E. E. Johnson, H. G. Ligget, Jr., C. A. McCarey, A. R. Moore, E. P. Moxey, Jr., Irving Orton, H. G. Pearce, E. Russell, I. L. Sessler, and B. L. Salomon.

TABLE I.—VARIATION IN THE NUMBER OF BLOOD CELLS AFTER EXERCISE.

Exp. no.	Blood count before exercise.		Kind of exercise taken.	Blood count after exercise.		Increase in cells.			
						Actual.		Percentage.	
	Red corpuscles.	Leucocytes.		Red corpuscles.	Leucocytes.	Red corpuscles.	Leucocytes.	Red corpuscles.	Leucocytes.
			Experiments made on athletes during actual competition.						
1	5,640,000	9,100	100 yard dash	7,040,000	14,100	1,400,000	5,000	24.8	55.0
2	5,500,000	9,240	120 yard hurdle race	6,680,000	12,320	1,180,000	3,080	21.5	33.3
3	5,600,000	8,900	½ mile run	6,600,000	12,500	1,000,000	3,600	17.9	40.4
4	5,620,000	8,820	1 mile run	6,400,000	13,660	780,000	4,840	13.9	54.9
5	5,550,000	9,200	2 mile run	6,040,000	18,100	490,000	8,900	8.8	96.7
6	5,600,000	8,100	Broad jump (6 jumps)	6,440,000	12,320	840,000	4,220	15.0	52.1
			Experiments made during preliminary training.						
7	5,610,000	8,740	50 yard and 100 yard dashes	6,720,000	13,600	1,110,000	4,860	19.8	55.6
8	5,520,000	8,600	100 yard dash	6,600,000	14,300	1,080,000	5,700	19.6	66.3
9	5,510,000	8,400	Two 50 yard sprints (full speed)	6,840,000	15,880	1,330,000	7,480	24.1	89.0
10	5,650,000	9,250	220 yard dash (full speed)	6,720,000	18,300	1,070,000	9,050	18.9	97.8
11	5,340,000	9,170	Two slow sprints of 100 yards each, and 220 yard dash at a rapid pace	5,900,000	11,100	560,000	1,930	10.5	21.0
12	5,560,000	8,720	440 yard run	6,600,000	11,890	1,040,000	3,170	18.7	36.4
13	5,600,000	8,330	2 starts and 440 yard run	6,490,000	11,620	890,000	3,290	15.9	39.5
14	5,520,000	8,320	660 yard run	6,360,000	14,780	840,000	6,460	15.2	77.6
15	5,410,000	7,900	660 yard run	6,520,000	11,600	1,110,000	3,700	20.5	46.8
16	5,500,000	7,970	4 starts, and two 440 yd. runs at a slow pace	6,800,000	11,320	1,300,000	3,350	23.6	42.0
17	5,410,000	7,960	440 yd. run and 660 yd. run, both at ½ speed	6,560,000	14,320	1,150,000	6,360	21.3	79.9
18	5,670,000	7,220	Two starts, and a ½ mile run at half speed	6,560,000	11,100	890,000	3,880	15.7	53.7
19	5,710,000	9,350	½ mile run at rapid pace	7,060,000	14,400	1,350,000	5,050	23.6	54.0
20	5,600,000	8,700	½ mile run at slow pace	6,700,000	12,400	1,100,000	3,700	19.6	42.5
21	5,630,000	8,800	½ mile run at full speed	6,720,000	12,800	1,090,000	4,000	19.4	45.4
22	5,510,000	9,430	¾ mile at fast pace	6,480,000	14,660	970,000	5,230	17.6	55.5
23	5,400,000	9,570	1 mile run (moderately fast)	5,800,000	14,000	400,000	4,430	7.4	46.3

24	5,750,000	8,690	1 mile run (slowly)	6,360,000	13,880	610,000	5,190	10.6	59.7
25	5,570,000	8,960	½ mile run (rapid pace), 1 mile run (slowly), and a few trials at hurdling	6,320,000	13,440	750,000	4,480	13.5	50.0
26	5,490,000	9,410	½ mi. run (rapid pace), 1½ mi. run (slow pace)	6,000,000	14,000	510,000	4,590	9.3	48.8
27	5,550,000	9,080	2 mile run (full speed)	6,340,000	14,000	790,000	4,920	14.2	54.2
28	5,500,000	8,250	2½ mile run (full speed)	6,280,000	13,880	780,000	5,630	14.2	68.2
			Experiments made upon the influence of long walks and bicycle rides.						
29	5,570,000	8,760	½ mile bicycle ride at rapid pace	6,510,000	12,610	940,000	3,850	16.9	44.0
30	5,480,000	9,270	½ mile bicycle ride at rapid pace	6,350,000	13,800	870,000	4,530	15.9	48.9
31	5,610,000	9,020	2 mile bicycle ride at rapid pace	6,230,000	12,960	620,000	3,940	11.1	43.7
32	5,440,000	8,930	2 mile bicycle ride at rapid pace	5,980,000	14,700	540,000	5,770	9.9	64.6
33	5,500,000	9,100	4 mile bicycle ride at rapid pace	6,090,000	13,380	590,000	4,280	10.7	47.0
34	5,470,000	9,110	4 mile bicycle ride at rapid pace	5,870,000	15,190	400,000	6,080	7.3	66.7
35	5,480,000	9,100	1 mile walk (rapidly)	6,400,000	15,000	920,000	5,900	16.8	64.8
36	5,590,000	9,060	2 mile walk (rapidly)	6,280,000	13,700	690,000	4,640	12.3	51.2
37	5,600,000	9,270	2½ mile walk (rapidly)	6,190,000	14,630	590,000	5,360	10.5	57.8
38	5,480,000	8,800	3 mile walk (rapidly)	6,260,000	15,700	780,000	6,900	14.2	78.4
39	5,400,000	8,930	3 mile walk (rapidly)	5,970,000	15,290	570,000	6,360	10.5	71.2
			Experiments made upon swimmers.						
40	5,480,000	10,540	100 yard handicap race	6,840,000	14,540	1,360,000	4,000	24.8	38.0
41	5,880,000	9,400	100 yard handicap race	6,960,000	14,700	1,080,000	5,300	18.4	56.4
42	5,700,000	9,200	100 yard exhibition swim against time[1] .	6,760,000	14,320	1,060,000	5,120	18.6	55.7
43	5,460,000	9,100	Water polo (3 minutes)[2]	6,920,000	18,600	1,460,000	9,500	26.7	104.4
44	5,500,000	8,800	Water polo (15 minutes)	6,280,000	16,440	780,000	7,640	14.2	86.8
45	5,680,000	10,000	Water polo (15 minutes)	6,450,000	16,000	770,000	6,000	13.6	60.0
46	5,720,000	8,350	4 times the length of the tank[3] at full speed	6,900,000	14,250	1,180,000	5,900	20.6	70.7
47	5,640,000	9,200	4 times the length of the tank at full speed	7,010,000	14,160	1,370,000	4,960	24.3	53.9
48	5,550,000	9,130	Tub race (twice the length of the tank) .	6,860,000	13,400	1,310,000	4,270	23.6	46.8
49	5,540,000	8,270	Twice the length of the tank at full speed	6,880,000	11,660	1,340,000	3,390	24.2	41.0
50	5,500,000	9,060	Twice the length of the tank at full speed	6,700,000	12,500	1,200,000	3,440	21.8	38.0

[1] This trial was one of the fastest ever made in the Houston Hall tank. [2] Pronounced shivering.

[3] The tank at Houston Hall is 46 feet 7 inches long and 17 feet 8 inches wide. The water is maintained at a temperature of 75° F., and is 3½ feet deep at one end of the tank and 9 feet deep at the other end.

III. Results.

1. **The normal blood count.** — By referring to Table I, page 390, it will be noticed that the normal blood counts were higher, in every case, than the normal commonly stated in the text-books. The blood counts of these athletic young men gave as an average 8800 leucocytes and 5,600,000 red corpuscles per cubic millimetre of blood, the ratio between leucocytes and red corpuscles being 1 : 636.

2. **Influence on the red corpuscles.** — Without exception, each one of the various forms of muscular exercise investigated caused an increase in the number of red corpuscles in the blood. As an average effect of the entire series of experiments an increase of 16.8 per cent was produced. The maximum increase of 1,460,000, or 26.7 per cent, was shown after the subject had played water polo for a period of three minutes, while the minimum increase of 400,000, or 7.3 per cent, was shown after the subject had ridden a bicycle a distance of four miles. By referring to the data for the experiments made on athletes during actual competition (see Experiments 1–5, Table I, page 390), it will be noticed that the greatest increase, one of 1,400,000, or 24.8 per cent, was produced by the muscular exertion accompanying a sprint of one hundred yards, whereas a run of two miles produced the minimum increase of 490,000 or 8.8 per cent. It will also be noticed that the running of the intermediate distances was accompanied by intermediate increases in the number of red corpuscles. In this series of experiments there was then an increase in the number of red corpuscles which was inversely proportional to the length of the exercise period. The same points are brought out in a somewhat less striking manner by the data from the experiments made while the subjects were doing their preliminary training. In these experiments the short dashes (fifty to two hundred and twenty yards) in general produced the greatest increase in the number of red corpuscles, while the long runs (one to two and one-half miles) were followed by the appearance of a decided minimum in the increased number of red corpuscles. In the whole number of experiments in which the influence of sprinting and running was studied (the experiments made during actual competition being included), an increase of 16.6 per cent in the number of red corpuscles was noted. By classifying these experiments as shown in Table II, page 394, we notice that the sprints and short runs, in which the distance travelled varied from fifty to six hundred and sixty yards,

produced an average increase of 20.3 per cent in the number of red corpuscles, whereas the longer runs in which the distance varied from one-half to two and one-half miles produced the much smaller average increase of 14.7 per cent.

The data for the experiments upon the influence of bicycle-riding show an average increase of 12.0 per cent in the number of red corpuscles. In agreement with the variations noted above, the comparatively short bicycle rides produced an increase of 16.4 per cent in the number of red corpuscles, and the longer bicycle rides produced the lower average increase of 9.7 per cent. Similar results were secured from the experiments upon the influence of walking.

Taken as a whole, the experiments upon the influence of swimming show the greatest average increase in the number of red corpuscles. This form of muscular exercise produced an average increase of 21.0 per cent as compared with the increase of 16.6 per cent after sprinting and running, that of 12.8 per cent produced by walking and that of 12.0 per cent secured as a result of bicycle-riding. As appears from the classification of the swimming experiments given in Table II, page 394, the short swims, continuing not longer than three minutes, produced the large increase of 22.5 per cent in the number of red corpuscles, while the long swims during which the subject was exceedingly active for a period of 15 minutes, produced the much smaller increase of 13.9 per cent. It is an interesting fact that the average increase of 22.5 per cent in the number of red corpuscles following these short swims was much larger than the increase produced by the short walks (16.8 per cent), short bicycle rides (16.4 per cent), or even by the sprints and short runs (20.3 per cent).

We have seen that each of the forms of muscular exercise studied has produced an increase in the number of red corpuscles in the blood. It is also apparent from a consideration of the various classes of experiments that strenuous physical exercise indulged in for a short period is followed by a more pronounced increase in the number of red corpuscles than is exercise of a like character but of greater duration. The first effect therefore of physical exercise of a more or less strenuous order is greatly to increase the number of red corpuscles in the blood, and this increase becomes gradually less pronounced as the exercise becomes more prolonged. The indications seem to point toward the possibility of a *decrease* in the number of red corpuscles as a result of violent muscular exercise sufficiently

prolonged. After securing an increase of 1,400,000 as the result of the running of a hundred yard dash, and the relatively insignificant increase of 400,000 after a two mile run, it is quite logical, we believe, to expect an actual decrease in the number of red corpuscles as a result of sufficiently prolonged muscular exertion. It is the author's intention to investigate this point further.

3. **Influence on the leucocytes.** — Each form of muscular activity studied caused an immediate increase in the number of leucocytes

TABLE II.

Average maximum and minimum increase in the number of red corpuscles and leucocytes after the various forms of muscular exercise.

Total no. of experiments.	Kind of exercise.	Percentage increase in number of blood cells.					
		Red corpuscles.			Leucocytes.		
		Av.	Max.	Min.	Av.	Max.	Min.
50	All the various forms investigated	16.8	26.7	7.3	57.0	104.4	21.0
28	Sprinting and running (including broad jump test)	16.6	24.8	7.4	55.8	97.8	21.0
6	Bicycle-riding	12.0	16.9	7.3	52.5	66.7	43.7
5	Walking	12.8	16.8	10.5	64.6	78.4	51.2
11	Swimming	21.0	26.7	13.6	59.2	104.4	38.0
12	Sprints and short runs (50–660 yards) . . .	20.3	24.8	15.2	59.9	97.8	33.3
14	Long runs ($\frac{1}{2}$–$2\frac{1}{2}$ miles) .	14.7	23.6	7.4	55.0	96.7	40.4
2	Short bicycle rides ($\frac{1}{2}$ mile)	16.4	16.9	15.9	46.4	48.9	44.0
4	Longer bicycle rides (2–4 miles)	9.7	11.1	7.3	55.5	66.7	43.7
1	Short walk (1 mile) . .	16.8	....	....	64.8	....	....
4	Longer walks (2–3 miles)	11.9	14.2	10.5	64.6	78.4	51.2
9	Short swims ($\frac{1}{2}$–3 minutes)	22.5	26.7	18.4	46.1	104.4	38.0
2	Long swims (15 minutes)	13.9	14.2	13.6	73.4	86.8	60.0

in the blood. Taking into account the entire fifty experiments, the average increase was 57.0 per cent. The maximum increase of 104.4 per cent was found after the three minute water polo period, and in this coincides with the occurrence of the maximum increase in the number of red corpuscles. The minimum increase of 21.0 per cent was produced by two one hundred yard dashes at a slow pace followed

after a short interval by two two hundred and twenty yard dashes at a more rapid pace.

By consulting Table II, page 394, it will be seen that the average increase in the number of leucocytes did not vary so greatly in the different forms of exercise as did the average increase in the number of red corpuscles. In the entire fifty experiments there is a variation from 46.1 per cent to 73.4 per cent in the leucocytes, and the relatively much greater variation from 9.7 per cent to 22.5 per cent in the red corpuscles. It will be noticed that the greatest variation in the average increase in the number of leucocytes in a series of experiments upon a single form of exercise is found in those experiments where the influence of swimming was studied. We observe that the short swims caused an average leucocytosis of 46.1 per cent, whereas the long swims produced an average leucocytosis of 73.4 per cent. It is interesting to note that the maximum and minimum average leucocytoses of the entire fifty experiments occurred as a result of the swimming tests, the long swims being followed by the large average leucocytosis and the short swims being followed by a much smaller average leucocytosis.

In Experiments 2–5, made during actual competition, there is an evident tendency of the leucocytes to increase parallel with the length of the exercise period. In these experiments the one hundred and twenty yard hurdle race caused a leucocytosis of 33.3 per cent, and the two mile run caused a leucocytosis of 96.7 per cent, while the distances between one hundred and twenty yards and two miles caused leucocytoses more or less *directly* proportional to the length of the race. It will be remembered that the increase in the number of red corpuscles in these same experiments was *inversely* proportional to the length of the exercise period. We thus see that the minimum increase in the number of red corpuscles was produced coincidently with the maximum leucocytosis. However, in no other series of experiments do the leucocytoses occur in this regular manner, and we are therefore inclined to consider the regular increase in the magnitude of the leucocytoses in Experiments 2–5 as possibly an accidental relation.

IV. Discussion.

1. Increase in the number of red corpuscles. — There are evidently at least six possible explanations for the appearance of an increased number of red corpuscles following muscular exercise. These may be stated as follows: —

1. Production of new corpuscles.

2. Concentration of the blood through increased urine formation and copious perspiration.

3. Concentration of the blood through increased evaporation in the lungs.

4. Concentration of the blood through vaso-motor contraction and rise in blood-pressure.

5. Sudden passage into the circulating blood of a large number of cells lying in various parts of the body and inactive before the time of the muscular exercise.

6. Passage of fluid from the blood to the active muscles.

The first explanation would be more plausible if our data were confined to the results of prolonged muscular exercise in which the increase in the number of red corpuscles was comparatively small. However, when we attempt to account for an increase of 1,400,000 red corpuscles (secured in a few seconds in the running of a hundred yard dash) by the formation of new cells the possibility seems rather remote. The second and third factors also might possibly account for the increase in the number of red corpuscles, provided the muscular exercise was sufficiently prolonged and the increase in the number of corpuscles was not too great. However, very often after running a hundred yard dash a participant will show practically no signs of sensible perspiration, and under such conditions an increase of approximately 25 per cent in the number of red corpuscles could hardly be due to the concentration of the blood through perspiration. Muscular work through the production of increased metabolic activity would very probably increase the flow of urine, and especially so if the urea content of the urine were high. But even under these conditions a considerable increase in the number of red corpuscles in the blood would occur only after prolonged exercise. Neither can the third possible explanation be accepted as sufficient to account for the large increase in the number of red corpuscles occurring immediately, as the accelerated rate of respiration causing increased evaporation in the lungs could hardly produce any marked concentration of the blood in a few seconds. For reasons similar to those already advanced, the fourth factor would seem inadequate as a full explanation for any immediate large rise in the number of red corpuscles.

The sudden accession of a large number of inactive cells, given as the fifth factor, affords a more logical explanation for at least the

greater portion of the large increase in the number of red corpuscles after a very short period of strenuous physical exercise. A similar explanation is advanced by Winternitz (*loc. cit.*) for the increase in the number of red corpuscles after cold baths, and also by Mitchell (*loc. cit.*) for the increase after massage. Willebrand on the contrary does not accept this view and gives data of experiments which indicate that the number of corpuscles per cubic millimetre at any given time is practically the same in the veins and the capillaries. Nevertheless, it seems highly probable that during rest there may be a considerable number of red corpuscles not actually needed in this resting period, but which quickly respond when suddenly called into play through a quickened respiration and increased muscular and metabolic activity. It is evident that the passage of the previously inactive corpuscles into the active circulation accounts for the immediate rise after muscular exercise. It would, however, seem that the entire reserve supply of corpuscles is very soon called into play, and that continued muscular exertion through a stimulation of the metabolic activities causes an increased destruction of corpuscles and a progressively decreasing corpuscle count, notwithstanding the tendency of increasing evaporation in the lungs, greater activity of the sweat glands, the passage of fluid from the blood to the working muscles (see below), etc., which act together to increase the specific gravity of the blood. This idea is confirmed by several tests made by Dr. Stewart in 1896 after two hour periods of violent physical exercise in the form of tennis-playing. Examinations of the blood were made before and after playing, and in nearly every instance this prolonged exercise resulted in a *decrease* in the number of red corpuscles in the blood. In line with these observations are those previously mentioned (page 392), in which a much greater increase was produced by running a hundred yard dash than by taking the more prolonged exercise incident to a two mile run. Those observations seemed to indicate that with sufficiently prolonged exercise a decrease in the number of red corpuscles would result.

It would also appear that the sixth factor, the passage of fluid to the active muscles, might reasonably be accepted as an explanation for a small part of the rise in the number of red corpuscles after not too prolonged muscular exercise. Ranke[1] tells us that working muscles are richer in water than resting ones, and that the greater

[1] RANKE: Tetanus, Leipzig, 1865. Quoted by LOEB: Archiv für die gesammte Physiologie, 1894, lvi, p. 270.

the activity of the muscle, the greater the water content. This increase in the water content of the working muscle, with the exception possibly of that part of the water which is produced by the oxidation of carbohydrates, would obviously reduce the amount of water in the blood and so would raise the specific gravity of the blood and incidentally increase the number of red corpuscles per cubic millimetre. It is on this theory that Willebrand (*loc. cit.*) explains the increase in the number of red corpuscles after muscular exercise. We are inclined to believe that this theory offers an acceptable explanation for a small portion of the first increase in the number of red corpuscles after muscular exercise. We are not inclined to accept it, however, as an adequate explanation of our observation that the increase in the number of red corpuscles was *inversely* proportional to the length of the exercise period. According to the theory of Ranke a two mile run should produce a much greater increase in the number of red corpuscles than a half mile run, when as a matter of fact we have repeatedly observed the reverse.

Finally, our idea regarding the relation existing between muscular exercise and the accompanying variation in the number of red corpuscles may be briefly stated as follows. The first effect of muscular work is a large increase in the number of red corpuscles, — an effect promoted possibly by more than a single cause, but which we believe to be principally due to the bringing into active circulation of large numbers of corpuscles which were inactive before the commencement of the muscular activity. In a short time all the available corpuscles are in service; and as the muscular activity continues unabated, the increasing metabolic processes cause the destruction of larger numbers of corpuscles than can be manufactured to replace them, and the very considerable increase in the number of red corpuscles noted in the first stage of the muscular activity becomes gradually smaller. At the same time vasomotor contraction, increased blood-pressure, accelerated urine formation, greater evaporation in the lungs, more copious perspiration from the skin, and the passage of fluid from the blood to the working muscles all tend to concentrate the blood and maintain the high blood count. As the exercise becomes more violent, these combined forces are unable to mask the effect of the ever-increasing destruction of red corpuscles, and a decrease in the number will be observed. It is thus apparently true that, although all the factors referred to operate in varying degree to increase the number of corpuscles per cubic millimetre, there is, from the very beginning

of muscular exercise, a constant decrease in the total number of corpuscles in the body, which reduces the blood count only when the decrease becomes sufficiently marked to overbalance the factors leading to an increase in the number of corpuscles per cubic millimetre.

2. Leucocytosis after muscular exercise. — Among the various conditions which might possibly cause a leucocytosis after muscular exercise we may mention the six already discussed in connection with the increase in the number of red corpuscles (page 396), and we would include another : (7) the changed distribution of the leucocytes followed by their accumulation in the peripheral circulation.

Inasmuch as the red corpuscles and leucocytes do not increase in the same ratio, the increase cannot be due primarily to concentration of the blood, and therefore we may eliminate factors (2), (3), (4), and (6). Explanation (1) may be rejected for the same reasons suggested in the discussion of the increase in the number of red corpuscles (page 396). The explanation for the greater part of the leucocytosis appears to be found in the changed distribution of the cells. The greatly increased rapidity of circulation would throw out into the general circulatory system great numbers of leucocytes from the large blood vessels and lymphatics of the interior, which, passing to the smaller arterioles and venules, where the circulation is slower, would show their well-recognized tendency to lag behind the general blood stream and produce an apparent leucocytosis of large dimensions.

V. Conclusions.

1. The normal blood count for healthy young men taking regular physical exercise was as follows: red corpuscles 5,600,000 per cubic millimetre, and leucocytes 8800 per cubic millimetre, the ratio between leucocytes and red corpuscles being 1 : 636.

2. The muscular exertion incident to running, walking, bicycle-riding, and swimming invariably caused an immediate increase in the number of red corpuscles per cubic millimetre and an accompanying leucocytosis.

3. The immediate increase in the number of red corpuscles per cubic millimetre of blood, after muscular exercise continuing for periods of a few seconds to about one hour, was inversely proportional to the length of the exercise period. The leucocytosis was inconstant.

4. The maximum average increase of 22.5 per cent in the number

of red corpuscles was caused by the short swims, the greatest increase (26.7 per cent) being secured after participation in water polo for three minutes.

5. The minimum average increase of 9.7 per cent in the number of red corpuscles occurred as a result of long bicycle rides, the smallest increase (7.3 per cent) being observed after a ride of four miles.

6. The maximum average increase of 73.4 per cent in the number of leucocytes followed the exertion incident to the long swims. The greatest individual increase (104.4 per cent), however, was caused by participation in water polo for three minutes.

7. The minimum average increase of 46.1 per cent in the number of leucocytes was observed after the short swims, the smallest increase (38.0 per cent) being caused by a hundred-yard swim.

8. The increase in the number of red corpuscles produced by muscular exertion is due primarily to the passage into the circulating blood of a large number of cells lying in various parts of the body and inactive before the time of the muscular exercise.

9. The leucocytosis following muscular exertion is due to the changed distribution of the leucocytes and their accumulation in the peripheral circulation.

THE RATE OF THE NERVOUS IMPULSE IN THE SPINAL CORD AND IN THE VAGUS AND THE HYPOGLOSSAL NERVES OF THE CALIFORNIA HAGFISH (*BDELLOSTOMA DOMBEYI*).

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Summary of Results.

THE rate of the nervous impulse in the spinal cord in the antero-posterior direction presents but small variations from one individual to another with a mean of 4.50 m. per second. This rate is probably through long tracts, or uninterrupted nerve fibres.

The postero-anterior rate in the spinal cord is subject to considerable variations with a mean of 2.50 m. per second.

The rate of nervous impulse in the motor fibres of the vagus to the musculature of the gill sacs presents but slight individual variations with a mean of 2.50 m. per second, the lowest rate in a peripheral motor nerve of vertebrates recorded under normal physiological conditions.

The rate in the motor fibres of the mandibular nerve is the same as the antero-posterior rate in the spinal cord, or 4.50 m. per second.

The rapidity of conduction of the impulse in the nerve stands in direct relation to the rapidity of the processes of contraction in the muscle innervated by the nerve; that is, the swifter the action of the muscle, the greater the rate of propagation of the impulse in the motor nerve supplying the muscle.

During my stay at the Hopkins Seaside Laboratory in the summer of 1902 I made some measurements of the rate of propagation of the nervous impulse in fishes, particularly in one of the Cyclostome or Marsipobranch fishes, *Bdellostoma*, commonly known as the California Hagfish. The Cyclostomes are the lowest of the Cordates, and since the classical work of Johannes Müller[1] the morphology

[1] MÜLLER: Vergleichende Anatomie der Myxinoiden, Berlin, 1834.

of this ancestral group of vertebrates has received considerable attention. The physiology of these primitive vertebrates has scarcely been touched, but from the small beginning that has been made, it would seem to be of no little interest to the comparative physiologist. Greene[1] has recorded the absence of cardio-inhibitory and cardio-accelerator nerves in *Bdellostoma*, the only case so far on record in the vertebrate phylum. My own measurements of the rate of propagation of the nervous impulse in the hagfish bring out the fact that the rate in the spinal cord is much lower than that in the ventral nerve-cord of some of the annelid worms, and that the rate in the peripheral motor nerves is the lowest recorded for any vertebrate and even lower than that in the motor nerves of some of the molluscs.

Previous researches touching the rate of the nervous impulse in the fishes have, with one exception, been confined to the nerve to the electrical organ of *Torpedo* and *Malapterurus*. Jolyet[2] has recorded a rate of 8 to 20 m. per second in the electrical nerve of *Torpedo*, while Schoenlein[3] found the rate in the same nerve to vary from 14 to 30 m. per second. Gotch and Burch[4] record a rate of 33 m. per second in the nerve fibres to the electrical organ of *Malapterurus*. Nicolai[5] has recently investigated the rate in the olfactory nerves of the Pike by recording the delays in the development of the action current. He found the rate in these nerves to be only 12 to 20 cm. per second.

In none of these researches was Helmholtz' method employed, although the fishes, both the Selachians and the Teleosts, offer serviceable nerve-muscle preparations. In view of the doubt that has recently been thrown on the negative variation as an infallible index of the nervous impulse, it is desirable to make use of the muscular response wherever possible.

I. The Rate of the Antero-Posterior Nervous Impulse in the Spinal Cord.

The measurements of the rate of propagation of the nervous impulse in the spinal cord of the hagfish involved nerve-muscle preparations

[1] Greene: This journal, 1901, vi, p. 318.

[2] Jolyet: Quoted from Schäfer's Text-book of Physiology, ii, p. 482.

[3] Schoenlein: Zeitschrift für Biologie, 1895, xxxi, p. 510; 1896, xxxiii, p. 425.

[4] Gotch and Burch: Philosophical transactions, London, 1896, B. p. 381.

[5] Nicolai: Archiv für die gesammte Physiologie, 1903, lxxxv, p. 65.

and experimental contrivances similar to those used in my work on the spinal cord of the snake.[1] For account of these and for a discussion of the sources of error in the measurements the reader is referred to that paper. It will here suffice to note the changes adapted to suit the case of the hagfish. It was found necessary to completely sever the head from the body prior to experimentation. If the cord was severed just behind the medulla, the animal would still continue to work its powerful jaw-muscles for some time, and this tended to keep the whole body in continuous swimming motion. The jaw-muscles are under the influence of the cranial nerves. Severing the spinal cord near the medulla does not therefore immediately put a stop to automatic or "conscious" functions of the brain in this animal any

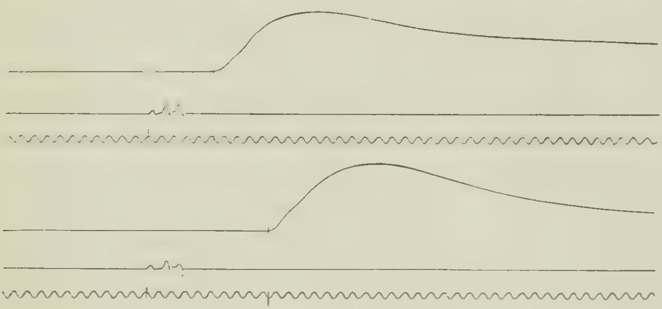

FIGURE 1. — Tracings from the contractions of the tail on the stimulation of the spinal cord near the medulla and about 8 cm. anterior to the level of the anus. Length of cord between central and peripheral electrodes: 36 cm. Transmission-time: 0.08 seconds. Rate: 4.50 m. per second. Time: 50 d. v. per second.

more than in the snake. The decapitated animal was placed flat on its side on the platform or board and secured by two strong steel pins that were pushed through the notocord into the board. This left the reacting tail portion flat and sidewise on the board. But the reactions to be recorded on the myograph were not actual shortening of the tail, but its side to side movements; and to exclude possible errors from the initial movement of the tail not being communicated to the lever if the contraction was on the side next to the platform, the end of the board was rounded off so that the tail, when relaxed and quiescent, curved downwards from the point of fixation posteriorly. By this contrivance the pull of the weighted (15 to 20 grams) recording lever kept the muscles of the upper side of the tail in

[1] CARLSON: Archiv für die gesammte Physiologie, 1904, ci, p. 23.

greater tension than on the under side, in consequence of which the initial movement of the tail following the stimulation of the spinal cord was always upwards.

The spinal cord of the hagfish has so little firmness that I did not succeed in exposing it for the application of the electrodes without injuring its conductivity. On slitting open the thick investing sheath or dura mater the spinal cord oozes out like a semi-fluid. Stimulating the cord through the thick investing sheath did not prove satisfactory. This prevented the taking of alternate records on proximal and distal stimulation, as was done on the preparations from the snake. In the hagfish the desired number of records of the distal stimulation of the cord had to be taken before the dissection was made for the proximal electrodes. But few records were taken from each preparation, to exclude the effects of fatigue and to render the records on distal stimulation strictly comparable to those on proximal stimulation. A slight increase in the delay and a decrease in the magnitude of the muscular response of the tail appear after a few reactions. This would have tended to make the calculated rate greater than the actual, if a great number of records had been taken on distal stimulation, thus fatiguing the preparation; but I do not think that this error figures materially in the results, because of the few records taken, and further because the preparations in which the spinal cord was stimulated at three levels (Table III, Experiments Nos. 12 to 17) exhibit no greater rate in the peripheral than in the central portion of the cord, which they obviously would if the fatigue effects were marked.

Contraction of the musculature of the tail does not always follow the stimulation of the cord with a single induced shock, just as was found to be the case in the snake. Two or three weak induced shocks following one another in rapid succession were therefore employed as stimulus.

The specimens, caught in traps and thus uninjured, were used on the same day that they were captured. The hagfish may be kept in the aquarium for months in apparently good condition, provided they are fed occasionally; but only specimens brought in the same day were used in order to avoid possible changes in the physiological conditions of the animals owing to changes in temperature and pressure and the lack of food.

The temperature of the aquarium varied from 13° to 15° C., that of the room from 17° to 19° C.

TABLE I.

Detail of Experiment No. 3, Table III. The spinal cord stimulated at two levels. Total latent time in seconds.

	Central.	Peripheral.
	0.170	0.100
	0.165	0.100
	0.178	0.093
	0.172	0.098
	0.170	0.100
	0.180	0.100
Aver.	0.172	0.098

Difference: 0.074. Length of cord: 38 cm.
Rate: 4.45 m. per sec.

TABLE II.

Detail of Experiment No. 12, Table III. The cord stimulated at three levels. Total latent time in seconds.

	Central.	Peripheral $_1$.	Peripheral $_2$.
	0.144	0.120	0.084
	0.150	0.130	0.089
	0.155	0.128	0.089
	0.149	0.124	0.090
Aver.	0.149	0.125	0.088

Difference, C-P_1: 0.024; P_1-P_2: 0.037. Length of cord, C-P_1: 14 cm.; P_1-P_2: 16 cm.
Rate, C-P_1: 5.74 m. per sec.; P_1-P_2: 4.32 m. per sec.

TABLE III.

Summary of the measurements of the antero-posterior rate in the spinal cord.

No. of experiment.	No. of pairs of records.	Transmission time in seconds.		Length of cord in cm.		Rate in m.	
1	4	0.070		32		4.54	
2	5	0.071		28		3.92	
3	6	0.074		33		4.45	
4	6	0.083		34		4.08	
5	3	0.058		30		5.16	
6	3	0.059		28		4.76	
7	3	0.071		28		3.94	
8	5	0.057		30		5.25	
9	4	0.078		32		4.19	
10	2	0.079		32		4.03	
11	6	0.048		17		3.53	
		C-P_1	P_1-P_2	C-P_1	P_1-P_2	C-P_1	P_1-P_2
12	4	0.024	0.037	14.0	16.0	5.74	4.32
13	3	0.041	0.040	18.0	16.0	4.39	4.00
14	3	0.031	0.047	12.0	23.0	3.86	4.87
15	4	0.030	0.040	15.0	17.0	4.99	4.23
16	3	0.023	0.037	14.5	16.0	6.23	4.32
17	4	0.027	0.045	16.0	20.0	5.92	4.44

Mean rate: 4.53 m. per sec. Standard deviation: 0.67 m.
Coefficient of variability: 0.14.

When taking into consideration the chances for errors involved in the experimental procedure, it must be admitted that the rate of propagation of the nervous impulse varies but little from one individual to another. The rate is comparatively low, being only about one-third that in the spinal cord of the snake. The rate of the nervous impulse in the hagfish is lower than that in the ventral nerve-cord of some of the annelid worms.[1] In *Glycera* and *Eunice*

[1] JENKINS and CARLSON: Journal of comparative neurology, 1903, xiii, p. 259.

the rate is the same as in the hagfish, or 4.50 m. per second, but in *Bispira* (one of the *Sabellidæ*) the rate is one-third greater than in the hagfish, or 7 m. per second.

It is possible that this rate of the nervous impulse in the spinal cord does not represent the rate of propagation in continuous nerve-fibres, but includes the delays at the junction or syapses of two or more systems of neurones. In the snake the records pointed to the conclusion that the antero-posterior paths in the spinal cord are long tracts. The spinal cord of the fishes contains long tracts in the Giant or Müllerian fibres. I know of no physiological evidence to the effect that these Giant nerve-fibres are motor, that is, paths for antero-posterior nervous impulses, although their anatomical relations may indicate that such is the case. The fact that the antero-posterior rate in the spinal cord is the same as the rate in the

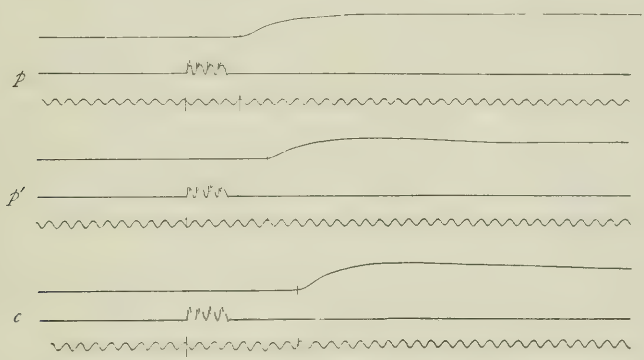

FIGURE 2. — Tracings from the contraction of the tail on the stimulation of the spinal cord at three levels, near the medulla (*c*), near the anus (*p*), and midway between these two points (*p′*). Length of spinal cord between *c* and *p′*: 18 cm.; between *p′* and *p*: 16 cm. Rate: in portion between *c* and *p′*, 4.50 m.; in portion between *p′* and *p*: 4.70 m. per second. Time: 50 d. v. per second.

peripheral motor nerves to muscles which show the same rapidity of contraction as the muscles of the trunk makes it probable that the rate in the cord represents the rapidity of the propagation of the nervous impulse in a system of continuous nerve fibres.

II. The Rate of the Postero-Anterior Nervous Impulse in the Spinal Cord.

For this set of experiments the decapitated animal was secured to the platform ventral side down. The contractions of the anterior

trunk muscles on stimulation of the spinal cord in the tail are much less regular and definite than the movements of the tail on stimulation of the spinal cord anteriorly. The anterior part of the hagfish exhibits also greater reflex activity than the tail end. When the tail is used for recording the muscular response, the lever may be

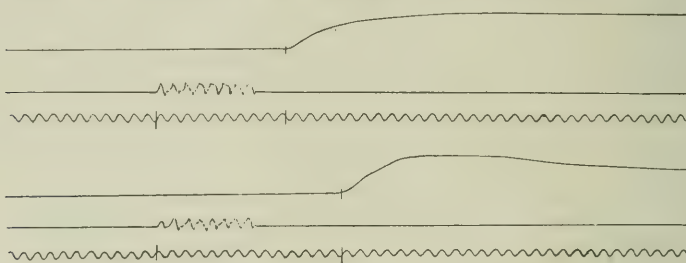

FIGURE 3.— Tracings of the contraction of the anterior part of the body on stimulation of the spinal cord in the tail and 20 cm. further anterior. Length of cord between distal and proximal electrodes: 20 cm. Transmission-time: 0.082 seconds. Rate: 2.40 m. per second.

weighted with 25 to 50 grams without the tension causing any movement, but in the experiments involving the anterior 6 to 8 cm. of the body as reacting portion, even a smaller weight of 10 to 20

TABLE IV.

Detail of Experiment No. 8, Table V. Total latent time in seconds.

Peripheral.	Central.
0.160	0.070
0.170	0.080
0.170	0.060
0.180	0.072
0.150	0.075
0.170	0.074
Aver. 0.166	0.072
Difference in the latent time: 0.094 seconds. Length of the spinal cord: 25 cm. Rate: 2.50 m. per second.	

grams generally keeps that portion of the body in a constant wiggling motion, which seriously interferes with the experiment.

If we exclude series 1, 2, and 15, the remaining ones show a fairly constant rate with a mean value of 2.50 m. per second, or but little more than one-half the antero-posterior rate. It would thus seem that it requires longer time for the nervous impulse to

TABLE V.

Summary of measurements of the postero-anterior rate in the spinal cord.

No. of experiment.	No. of pairs of records.	Transmission-time in seconds.	Length of cord in cm.	Rate in m.
1	6	0.350	26	0.74
2	7	0.309	26	0.83
3	3	0.115	24	2.08
4	7	0.133	25	1.87
5	2	0.090	25	2.77
6	5	0.070	20	2.86
7	7	0.120	32	2.65
8	6	0.094	25	2.50
9	3	0.110	28	2.54
10	3	0.120	25	2.07
11	2	0.120	28	2.32
12	5	0.085	19	2.47
13	2	0.100	26	2.60
14	3	0.080	24	3.00
15	4	0.300	24	0.80
Mean rate: 2.13 m. per second. Excluding Experiments 1, 2 and 15: 2.47 m. per second.				

travel from tail to head than from the head to tail in the spinal cord of this animal. But this does not necessarily mean that the rate of transmission in the nerve fibres themselves is actually different; for the centripetal conducting paths in the cord may be more complex than the centrifugal conducting paths, and the longer trans-

mission-time due to this greater complexity. The fact that the contraction of the trunk muscles is more readily produced posterior than anterior to the point of stimulation of the spinal cord favors this explanation.

Nothing in the experimental conditions aids in the interpretation of the three series (Nos. 1, 2, 15) which show such a great departure from the rest. The great increase in the transmission-time may, of course, be due to accidental injury to the cord either in the capture or in preparing the specimens for the experiments. But this would hardly account for the fact that the rate is equally low in the three specimens, or 74.83 and 80 cm. respectively.

III. The Rate of the Nervous Impulse in the Vagus Nerve.

The motor fibres of the vagus nerve make connection with two systems of muscles, either of which may serve as the reacting muscle in measuring the rate of propagation of the impulse in the nerve.

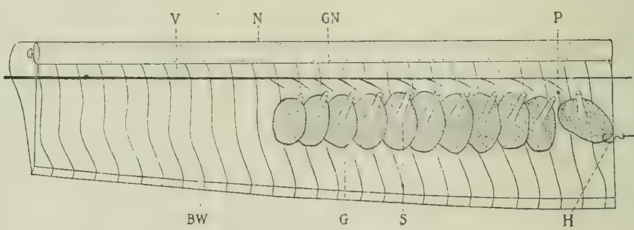

Figure 4. — Two-thirds natural size. *Bdellostoma dombeyi.* Right anterior half of body, showing relation of the vagus to the gill sacs. *BW*, body wall; *G*, gills; *GN*, gill nerves; *H*, hook serving for attachment of the thread to the recording lever; *N*, notocord; *P*, pin by which one edge of the gill sac was fixed to the board; *S*, sinus connecting the gill sac with the œsophagus; *V*, vagus nerve.

These are the musculature of the gill sacs and the *constrictor cardiæ.* The relation of the vagus to the branchial baskets or gill sacs is shown in Fig. 4. The number of these gill sacs in the hagfish varies from eleven to thirteen pairs. The walls of the gill sacs are provided with a thin layer of striated muscle, innervated by branches from the vagus nerve of the same side. In these experiments one of the posterior of the several gill sacs served for contraction. In the largest specimens this gives a distance of nerve between the gill sac and the exit of the vagus through the cranial cartilage of from 12 to 14 cm. The vagus is easily exposed and freed from the adjoining adipose and

connective tissues. The shortcomings of this nerve-muscle preparation consist in the small, feeble, and relatively slow contraction of the musculature of the gill sac. The maximal contraction of the gill sac amounts to about 1 mm. The recording lever has to be very light and delicately balanced, as the strength of the musculature is small; and this prevents any great magnification of the amplitude of the contraction. The muscle, although striated, is relatively slow in its action. As registered by the lever, it requires 0.12 second to reach the maximal contraction of 1 mm., a longer period than the whole contraction time of the frog's gastrocnemius, while both contraction and relaxation of the gill sac require no less than 0.3 to 0.4 second. This makes necessary a slow motion of the recording surface, so slow, in fact, that the delay of the muscular response in fractions of a second cannot be measured with any accuracy further than to the second decimal. But because of the very slow rate of propagation of the impulse in the nerve, together with the long distance of nerve obtainable between the distal and the proximal electrodes, smaller fractions may be left out without influencing the results.

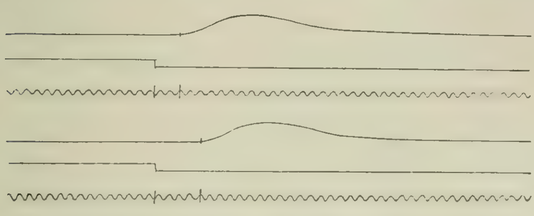

FIGURE 5. — Tracings of the contractions of the gill sac on the stimulation of the vagus near the cranium and near the gill sac. Length of nerve between the central and peripheral electrodes: 11 cm. Transmission-time: 0.046 second. Rate: 2.40 m. per second. Time: 50 d. v. per second.

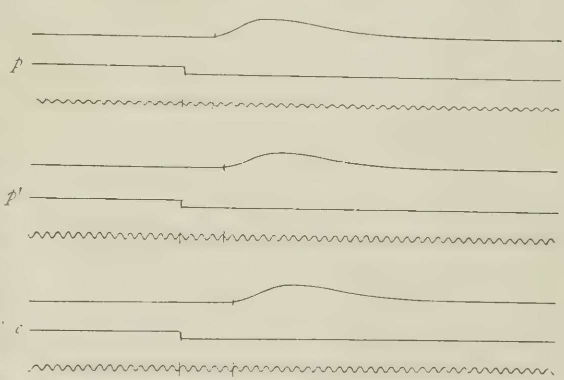

FIGURE 6. — Tracings of the contraction of the gill sac on the stimulation of the vagus successively at three points. Length of nerve between central and peripheral′ electrodes: 4 cm.; between peripheral′ and peripheral: 6 cm. Rate: *c* to *p′*, 2.66 m.: *p′* to *p*, 2.60 m. per second. Time: 50 d. v. per second.

TABLE VI.

Detail of Experiment No. 4, Table VIII. The nerve stimulated at two levels. Total latent time in seconds.

Central.	Peripheral.	Central.	Peripheral.
0.110	0.070	0.102	0.060
0.100	0.070	0.100	0.070
0.100	0.070	0.100	0.070
0.105	0.068	0.100	0.060
0.100	0.070	0.108	0.060
0.104	0.070	0.100	0.060
0.107	0.073	0.110	0.070
0.112	0.070	0.110	0.070

Average latent time, central: 0.104 second. Peripheral: 0.067 second. Difference: 0.037 second. Length of nerve: 9 cm. Rate: 2.43 m. per second.

TABLE VII.

Detail of Experiment No. 11, Table VIII. The nerve stimulated at three levels. Total latent time in seconds.

Central.	Peripheral$_1$.	Peripheral$_2$.
0.100	....	0.060
0.100	....	0.060
0.100	0.085	0.062
0.100	0.082	0.065
0.100	0.085	0 060
0.100	0.085	0.060
Aver. 0.100	0.084	0.061

Difference, C-P$_1$: 0.016; P$_1$-P$_2$: 0.023. Length of nerve, C-P$_1$: 4 cm. P$_1$-P$_2$: 6 cm. Rate, C-P$_1$: 2.50 m. per sec.; P$_1$-P$_2$: 2.58 m. per second.

The *constrictor cardiæ*, a system of oblique and circular muscle surrounding the œsophagus at its junction with the stomach, is stronger than the musculature of the gill sacs, but this muscle is not so easily isolated for the purposes of these experiments.

Instead of removing the vagus, together with one of the gill sacs, from the body of the animal for the experiments it was found more convenient and quite as accurate to cut the animal in two just behind

TABLE VIII.

Summary of the measurements of the rate in the vagus nerve.

No. of experiment.	No. of pairs of records.	Transmission-time in seconds.		Length of nerve in cm.		Rate in m.	
1	14	0.034		9.0		2.64	
2	5	0.025		6.0		2.40	
3	30	0.058		11.0		1.89	
4	16	0.037		9.0		2.43	
5	12	0.040		11.5		2.87	
6	7	0.048		10.0		2.08	
7	9	0.030		7.5		2.48	
8	14	0.052		11.5		2.18	
9	7	0.040		11.0		2.75	
10	6	0.023		5.5		2.38	
		$C-P_1$	P_1-P_2	$C-P_1$	P_1-P_2	$C-P_1$	P_1-P_2
11	5	0.016	0.023	4.0	6.0	2.50	2.58
12	4	0.021	0.019	4.0	4.5	2.14	2.36
13	6	0.028	0.024	5.0	4.5	1.78	1.87
14	4	0 021	0.019	5.5	6 0	2.36	3.15

Mean rate: 2.40 m. per second. Standard deviation: 0.35 m.
Coefficient of variability: 0.14.

the hindmost gill sac, fix the anterior portion dorsal side down on the platform used for the experiments on the spinal cord, expose the visceral cavity by an incision in the ventral median line, and having pinned the body walls to either side, free the vagus nerve down to the gill sac desired, but leave the gill sac *in situ*, the thread to the

recording lever passing over a friction wheel at the end of the stand just as in the experiments on the spinal cord. This preparation is represented in the diagram in Fig. 4.

As a control of the measurements, as well as with the view of obtaining data bearing on the question of acceleration or retardation of the rate with the distance of propagation, the nerve was stimulated at three levels in four of the preparations (Table VIII, Nos. 11 to 14).

The preparation is not readily fatigued, 30 pairs of records being obtained in one case without any obvious increase in the delay of the muscular response.

The break-induced shock is a more efficient stimulus to the motor fibres in the vagus than the make shock; the former was therefore used throughout the experiments.

IV. The Rate of the Nervous Impulse in the Mandibular Nerve.

The musculature of the lower jaw of the hagfish is a very complicated apparatus peculiar to the Marsipobranch group. It has been described by Müller in the afore-cited work and more recently by Ayers and Jackson.[1] There are two retractor muscles of the lower jaw, a circular and longitudinal. The longitudinal retractor muscle and its innervation are represented in Fig. 7. The muscle is divided in the median line into a right and a left half, tapering anteriorly where they fuse into one in joining the cartilaginous tendon of the jaw. At the posterior end the two muscles are separated by a wedge-shaped transverse (dorso-ventrally) muscle (Fig. 7, *tm*). The strongly developed circular muscle completely surrounds the longitudinal muscle, except at its posterior end. The longitudinal retractor muscle is innervated by two branches from the combined root of the fifth and the seventh cranial nerves.[2] The exit of these branches through the cranium is just in front of the lateral cartilage, where they may be exposed very readily by removing the skin, together with one of the muscles of the head. The branch from either side takes a posterior, ventral, and median direction to unite in one nerve at the anterior edge of the circular retractor muscle. This nerve continues posteriorly within the cavity formed by the circular muscle, adhering

[1] Ayers and Jackson: University of Cincinnati Bulletin, No. 1, 1900.

[2] Allis: Anatomischer Anzeiger, 1903, xxiii, p. 259.

closely to the dorsal wall, and enters the longitudinal muscle dorsally near the posterior end, as indicated in Fig. 7. The nerve is readily isolated for the purposes of the experiment; the muscle is strong enough to raise a relatively heavy weight; and in the largest specimens a length of nerve of from 9 to 10 cm. is obtainable, which together make this nerve-muscle preparation admirably suited for measuring the rate of propagation of the impulse in the nerve by the ordinary graphic method.

FIG. 7. — One-half natural size. *Bdellostoma dombeyi.* Diagram of the retractor mandibular muscle with its innervation, ventro-lateral view. *LMN*, left mandibular nerve; *RMN*, right mandibular nerve; *LRM*, left half of retractor muscle; *RRM*, right half of retractor muscle; *T*, tendon connecting with the lower jaw; *TM*, transverse muscle.

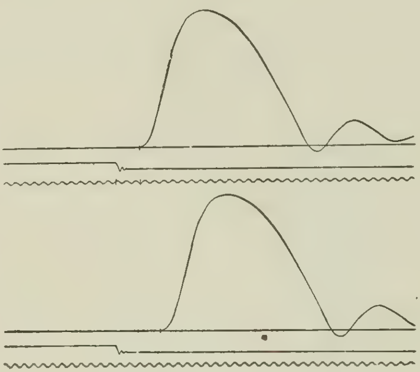

FIG. 8. — Reduced one-half. Tracings of the contraction of the retractor mandibular muscle on stimulation of the mandibular nerve near the cranium and near its entrance into the muscle. Length of nerve between central and peripheral electrodes: 9.5 cm. Transmission-time: 0.021 second. Rate: 4.50 m. per second.

The rate of propagation of the impulse in the motor fibres of the vagus is the lowest that has been recorded in a peripheral motor nerve in the vertebrates under normal physiological conditions. It is much lower than that in the peripheral motor nerves of some of the invertebrates (Table XI). The rate in the mandibular nerve is nearly twice that in the vagus, and there is a corresponding difference between the rapidity of contraction of the retractor muscle and the musculature of the gill sacs, the contraction-time of the former being 0.18 second, that of the latter 0.45 second. The anteroposterior rate in the spinal cord is the same as that in the mandibular nerve, and the contraction-time of the trunk muscles is also nearly

the same as that of the retractor muscle, which makes it probable that the antero-posterior rate in the cord as determined by this method of measurement represents the rapidity of propagation of the impulse through continuous nerve fibres.

The peripheral nerves as well as the nerve fibres in the spinal cord of the hagfish are non-medullated. I have made no chemical tests for the presence of myelin in the spinal cord and the nerves, but the

TABLE IX.

Detail of Experiment No. 1, Table X. The nerve stimulated at two levels. Total latent time in seconds.

Distal.	Proximal.	Distal.	Proximal.
0.046	0.026	0.044	0.026
0.044	0.026	0.042	0.024
0.042	0.023	0.045	0.025
0.045	0.025	0.046	0.026
0.042	0.025	0.047	0.027
0.044	0.023	0.046	0.026
0.042	0.024	0.047	0.026
0.046	0.026	0.047	0.026
0.045	0.026	0.046	0.027

Average latent time, distal: 0.044 second. Proximal: 0.025 second.
Difference in the latent time: 0.019 second. Length of nerve: 9.5 cm.
Rate: 495 m. per second.

osmic acid and other histological methods fail to show the presence of a medullary sheath. This condition appears to be characteristic of the Marsipobranchs. Reissner[1] records the absence of medullary sheaths in the spinal cord and in the peripheral nerves of *Petromyzon*, which has since been confirmed by Ransom and Thomson.[2] Nansen[3] found the same condition to obtain in Myxine.

The records from the four preparations in which the vagus nerve was stimulated at three levels (Table IV, No. 11 to 14) afford a con-

[1] Reissner: Archiv für Anatomie und Physiologie, 1860, p. 545.

[2] Ransom and Thomson: Zoologischer Anzeiger, 1886.

[3] Nansen. Quoted by Retzius: Biologische Untersuchungen, n. f., ii, p. 47.

clusive check on the other measurements, but they are not conclusive on the question of acceleration or retardation of the rate with the distance of propagation.

TABLE X.

Summary of the measurements of the rate in the mandibular nerve.

No. of experiment.	No. of pairs of records.	Transmission-time in seconds.	Length of nerve in cm.	Rate in m.
1	18	0.019	9.5	4.95
2	4	0.007	3.5	5.00
3	10	0.025	10.0	4.00
4	6	0.010	4.5	4.50
5	6	0.012	6.5	5.39
6	11	0.038	10.5	3.50
7	5	0.024	10.0	4.20
8	6	0.020	8.0	4.00
9	10	0.019	9.5	4.94
10	6	0.013	5.0	3.85
11	6	0.011	4.0	3.64

Mean rate: 4.40 m. per second. Standard deviation: 0.50 m.
Coefficient of variability: 0.11.

The relation between the rate of propagation of the impulse in the vagus and the mandibular nerves and the rapidity of the processes of contraction in the muscles innervated by these nerves suggests that *the rapidity of the processes of conduction in the nerve stands in direct relation to the rapidity of the processes of contraction in the muscle.* This is brought out even more strongly by the series of comparations in Table XI. To be sure, the increase in the contraction-time does not proceed *pari passu* with the decrease in the rate of propagation in the nerve to the extent that a numerical ratio can be established. For example, the contraction-time of the flexor muscle of the chelæ in the lobster is more than twice that of the mantle muscle of the squid, yet the rate in the lobster nerve is greater than that in the squid nerve. The flexor muscle of the chelæ attains its maximal degree of shortening within less than 0.10 second, but the relaxation

is very gradual. This gradual relaxation is less marked in the muscles of the other ambulacral appendages. The comparison between the nerve and the muscle should perhaps be made, not with

TABLE XI.

Comparation between the contraction-time of the muscle and the rate of propagation of the impulse in the nerve.

Species.	Muscle.		Nerve.	
	Muscle.	Contraction-time in seconds.	Nerve.	Rate of the impulse in m.
Frog . .	gastrocnemius	0.10	sciatic (medullated)	27.00
Snake . .	hyoglossus	0.15	hypoglossus (medullated)	14.00 [1]
Lobster . (*Homarus*)	flexor of chelæ	0.50	first ambulacral (non-medullated)	6.00 [2]
Squid . . (*Loligo*)	mantle (fin)	0.20	mantle nerve (non-medullated)	4.50 [3]
Hagfish .	retractor of jaw	0.18	mandibular (non-medullated)	4.50
Hagfish .	gill sac	0.45	vagus (non-medullated)	2.50
Octopus .	mantle	0.50	pallial (non-medullated)	2.00 [3]
Slug . . . (*Limax*)	foot	4.00	pedal (non-medullated)	1.25 [3]
Sea hare . (*Pleurobranchæa*)	foot	10.00	pedal (non-medullated)	0.75 [3]
Slug . . . (*Ariolimax*)	foot	20.00	pedal (non-medullated)	0.40 [3]

[1] Carlson: Archiv für die gesammte Physiologie, 1904, ci, p. 23.

[2] Fredericq and Vandevelde: Bulletins de l'Académie Royale de Belgique, 2 sér., xlvii.

[3] Jenkins and Carlson: This journal, 1903, viii, p. 251.

the whole contraction-time of the muscle, but with the time required to reach the maximal degree of shortening.

This direct relation between the rapidity of conduction in the motor nerves and the rapidity of contraction in the muscle favors the view that a similar relation obtains between the processes of conduction in the secretory nerves and the processes of secretion in the glands. The rate of the nervous impulse would thus constitute a measure of the relative rapidity of the metabolic processes in muscle and gland.

THE RELATION OF IONS TO CILIARY MOVEMENT.

By RALPH S. LILLIE.

[*From the Marine Biological Laboratory, Woods Holl, Mass.*]

THE addition of small quantities of salts with bivalent or trivalent cations (including those of many otherwise poisonous heavy metals) to pure solution of single salts (chiefly sodium salts, also salts of lithium, potassium, and ammonium) has been found to counteract the toxic action of such solutions to a degree varying with the concentration and chemical nature of the added cation. Thus Fundulus eggs will not form embryos in pure solutions of sodium chloride, nitrate, or acetate, but on the addition of any one of the following cations in appropriate quantity, development and embryo-formation become possible: Ca, Mg, Sr, Ba, Co, Fe'', Zn, Mn, Pb, Al, Cr.[1] So also voluntary muscle retains its irritability distinctly longer in solutions of sodium, potassium, lithium, or ammonium chloride containing certain of the above cations, than in the pure solution alone.[2] The capability of acting thus *antitoxically* is apparently peculiar to cations: addition of equivalent quantities of various anions has in general been found to be without such action.[3] Hence the sign of the ionic charge is evidently an important factor in the production of these effects, indicating that the phenomenon is fundamentally electrical in nature; this is further indicated by the marked differences of action exhibited by cations of different valence,

[1] LOEB, J.: Archiv für die gesammte Physiologie, 1901, lxxxviii, p. 68; LOEB and GIES: *Ibid.*, 1902, xciii, p. 246; LOEB: This journal, 1902, vi, p. 411. Fe''', Cd, Cu, and Hg gave negative results; Th, Ni, and UO_2, only faint indications of antitoxic action.

[2] NEILSON: This journal, 1902, vii, p. 405.

[3] Miss MOORE, however (This journal, 1902, vii, p. 1), found that sodium sulphate could apparently counteract the injurious action of pure sodium chloride on lymph-hearts and skeletal muscle. Other anions than SO_4 failed to give the effect which thus appears to be special to SO_4, and not a general property of all anions.

— trivalent ions acting antitoxically in far more dilute concentrations than bivalent ions, and these again requiring for an equivalent antitoxic action far lower concentrations than monovalent ions. These relations recall the proportions lately found to exist between equicoagulative concentrations of mono-, bi-, and trivalent ions in the precipitation of colloidal solutions by electrolytes.[1] They suggest that a certain subdivision or *state of aggregation* of the protoplasmic colloids is a condition of normal physiological activity, and that ions affect vital processes largely by producing alterations in this normal state, causing in some cases coalescence, in others still further comminution of the colloidal particles.

It is important to determine if these relations apply also to other forms of vital activity, and especially to the different forms of protoplasmic contractility. Contractility is a property intimately dependent on the presence of ions.[2] The exact rôle played in the contractile tissues by ions is unknown; but it seems probable that in addition to their influence on the state of colloidal aggregation, *the ionic charges may effect alterations of surface-tension at the various semi-permeable surfaces*, — either within the cell (as the surface of the fibrillæ in muscle) or at the external boundary of the cell or its appendages (*e. g.*, cilia), — and so give rise to movements in a manner analogous to that of the ions in the capillary electrometer. It is known that many cell-membranes act as semi-permeable membranes toward certain ions;[3] the possibility therefore exists that differences of ionic concentration may arise on opposite sides of such a cell-membrane, of such a kind that the tension of the adjoining protoplasmic surface, or possibly of the cell-membrane itself, may undergo corresponding alterations leading to change of form, — contraction or relaxation, as the case may be. The same conception may possibly apply, with appropriate modifications in each special instance, to all of the forms of contractility.[4] Differences of permea-

[1] *Cf.* the papers of SCHULZE, PICTON and LINDER, HARDY, VAN BEMMELEN. For reference, *cf.* HOEBER'S "Physikalische Chemie der Zelle und der Gewebe," Leipzig (ENGELMANN), 1902, p. 161.

[2] *Cf.* especially the papers of J. LOEB, who has especially emphasized the importance of this relation of ions to contractility.

[3] *Cf.* papers of HAMBURGER, OVERTON, GRYNS, KOEPPE. For references, *cf.* HÖBER, *loc. cit.* *Cf.* also KOEPPE: Archiv für die gesammte Physiologie, 1903, xcix, p. 33.

[4] In Biological Bulletin, 1903, iv, pp. 169–178, I have given a more detailed hypothesis to account for the special case of mitotic cell-division.

bility may possibly explain why different contractile tissues react differently toward the same ions.[1]

EXPERIMENTAL.

The following experiments were performed at Woods Holl during the summer of 1903, and are partly in continuation of studies already published.[2] In the two preceding papers I have described certain facts indicating that a special state of aggregation of the ciliary substance is necessary for continued movement. Pure $\frac{m}{2}$ NaCl rapidly liquefies cilia, and hence arrests movement. Liquefaction is retarded or prevented by the addition of traces of calcium or magnesium salts (especially the latter), with the result that movement may continue without interruption for many hours in such solutions. Hence the cations calcium and magnesium, in preventing disintegration and enabling movement to continue, act antitoxically in the above sense. In the experiments described below, I have examined the action of a large number of ions of varying valence and chemical character, with especial reference to this power of preventing ciliary disintegration and sustaining the movement.

The ciliated organism employed in all of the following experiments was the bitrochal ciliated larva of *Arenicola* described in the first paper of this series. Arenicola larvæ are readily obtained in large quantity by rearing: they have the advantage of possessing an extreme constancy and definiteness of reaction; incidentally their pronounced phototaxis affords a means of freeing them almost completely from sea-water before transfer to the solutions. Most of the following experiments were made in watch-glasses of 10 c.c. capacity. The usual procedure is as follows: The organisms are allowed to collect in a mass on the light side of the watch-glass. The sea-water is then drawn off, and its last adhering traces are removed by filter paper. The solution is then added, and observation is made at once under the microscope. The slight traces of sea-water adhering to the organisms — at most one volume in several hundred of solution — do not affect the result perceptibly, as I have assured myself by repeated control experiments. The response is, as a rule, instantaneous, and very definite and constant.

[1] Why, for instance, muscle loses contractility instantly in solutions of MgCl or KCl, while cilia continue their activity unchecked. *Cf.* R. LILLIE: This journal, 1901, v, p. 56.

[2] R. LILLIE: This journal, 1901, v. p. 56; 1902, vii, p. 25.

Action of Pure Solutions of Various Salts.

Solutions of pure sodium salts all have an effect closely resembling that already described for sodium chloride. The following sodium salts have been used in pure $\frac{m}{2}$ solution: chloride, bromide, fluoride, nitrate, acetate, sulphate, tartrate, phosphate, citrate. The typical result for all solutions is an almost immediate cessation and total or partial liquefaction of the cilia; in a few instances, cilia may remain feebly active for a minute or more, exceptionally for two to three minutes. No distinct differences of action are seen with different salts; liquefaction of cilia with consequent cessation of movement is thus the typical action of all sodium salts in pure solution; the effect is apparently specific to the sodium ion, in the absence of other and counteracting ions. Lithium chloride acts similarly to sodium chloride.

Ammonium and potassium salts are less injurious to ciliary movement than sodium and lithium salts, but act more destructively on muscular contractility. In $\frac{m}{2}$ KCl, KBr, KNO_3, K_2SO_4, cilia remain active for considerable periods of time: at first the organisms swim actively, but the body is rigid from the absence of muscular contraction, and the swimming is irregular, soon leading to the irregular aggregation or "clumping" described in my earlier papers. Ciliary movement may continue for an hour or more. Little difference appears between different solutions, — in all, the typical action of the potassium ion is seen, allowing ciliary movement to continue, but powerfully antagonizing muscular contraction.

In pure solutions of ammonium salts (chloride, nitrate, sulphate) cilia also remain active for some time, although not so long as with potassium salts, nor are muscular contractions so quickly arrested as in potassium solutions. Ammonium seems intermediate between sodium and potassium in its action, though generally resembling potassium rather than sodium.

Cilia remain active longer in pure solutions of certain chlorides with bivalent cations, than in any other media containing only one salt. Solutions of magnesium chloride and manganese chloride are the most favorable; with concentrations ranging from $\frac{m}{2}$ to $\frac{m}{4}$ movement may continue for several hours in magnesium chloride solutions, and for one to two hours in manganese chloride. Muscular contraction is quite impossible in these solutions. Calcium and strontium

chlorides are less favorable to ciliary movement than are the above two salts, but destroy muscular contractility less rapidly. Barium chloride is the most poisonous of the alkaline earth chlorides; yet in $\frac{m}{2}$ solutions cilia may remain feebly active for thirty minutes or more; muscular contractions are prevented from the first as with magnesium. Barium appears to resemble magnesium rather than calcium or strontium. It is remarkable that the chlorides of several heavy metals besides manganese are less injurious to ciliary movement in pure solution than is sodium chloride. Thus in $\frac{m}{2}$ or $\frac{m}{3}$ $CoCl_2$ or $NiCl_2$ solutions movement may continue for so long as twenty minutes; even in pure $\frac{m}{3}$ $CdCl_2$ solutions, movement may continue for several minutes. In solutions of cupric chloride, zinc sulphate, and lead acetate, liquefaction and cessation of movement occur almost immediately. Salts of trivalent metals (aluminium chloride, chromium sulphate, ferric chloride) also produce in all cases an instantaneous liquefaction and cessation of movement.[1]

Can Anions Counteract Toxic Influence of Sodium Salts?

In the following series, sodium salts with different anions were added to solutions of sodium chloride.

1. 100 vol. $\frac{m}{2}$ NaCl (control).
2. 98 " " " 2 vol. $\frac{m}{2}$ $NaNO_3$.
3. 98 " " " 2 " " NaBr.
4. 98 " " " 2 " " $NaCOOCH_3$.
5. 98 " " " 2 " " Na_2SO_4.
6. 96 vol. $\frac{m}{2}$ NaCl 4 vol. $\frac{m}{4}$ $Na_2C_2O_4$.
7. 98 " " " 2 " $\frac{m}{2}$ Na_2 tartrate.
8. 98 " " " 2 " " Na_3 citrate.
9. 98 " " " 2 " " Na_2HPO_4.

In none of these solutions was there evident any distinct counteracting influence, liquefaction and cessation of movement appearing promptly in all, as with pure sodium chloride. The results are thus uniformly negative. Weakly alkaline solutions of sodium salts also act destructively on cilia. Apparently anions, whatever their valency and chemical character, do not in the above concentrations appreciably counteract the injurious action of the pure solution; whereas equivalent quantities of cations have, in the majority of instances, a most pronounced antitoxic influence, as will appear below. Anions thus present a contrast to cations in respect to antitoxic power.

[1] It is a matter of some interest that muscular contraction is impossible in *al* solutions of heavy metal salts, — another indication of the essential difference between the respective physico-chemical conditions of muscular and ciliary movement.

This conclusion was also reached by Loeb in the papers cited above. On account of the negative outcome of the above, and other series of experiments with anions, relatively few further determinations of this kind were made.

Antitoxic Action of Cations.

To a certain degree cations appear to act antitoxically by simple virtue of their electrical positivity. This is seen in the fact that practically all cations hitherto tested, with the exception of a few of the most toxic (mercury, silver), exhibit more or less antitoxic action, though in certain instances, as with Cu, UO_2, and Fe''', the effect is slight and obscured by the directly toxic action of the ion. Valence is also a factor in antitoxic action, as Loeb has demonstrated.[1] But that other factors must also enter, is seen in the greatly varying effectiveness of different cations having the same valence.

The interesting hypothesis of Mathews[2] that the physiological action of an ion, and so its toxicity, may be largely dependent on the readiness with which the ionic charge is set free (the "fixation-intensity" of the charge), in other words, on the solution-tension of the ion,[3] may serve in part to explain differences in the toxic and anti-toxic action of different cations. It is certain that those cations which have the lowest solution-tension, *i. e.*, are discharged most readily, as Ag, Hg, Pt, Au, Pb, H, Cu, are among the most toxic: and that Mn and Mg, whose solution-tensions are among the highest, are remarkably non-toxic.[4] Mathews points out that a general parallelism appears to hold between solution-tension and toxicity, though to this there are several exceptions, as Zn and Cd, which are more poisonous than their position in the solution-tension series can explain. But that still other factors than the above enter into the case, is seen in the fact that the presence of *two* cations, in addition to the sodium chloride, may exert an incomparably greater influence than that of either

[1] Loeb, J.: *Loc. cit.*

[2] Mathews, A. P.: This journal, 1904, x, p. 290.

[3] For a table of the comparative solution-tensions of a number of ions, see Nernst: Theoretische Chemie, 3d ed. p. 675. See also Wilsmore and Ostwald: Zeitschrift für physikalische Chemie, xxxvi, 1901, p. 92; and Mathews: *Loc. cit.*

[4] The coagulative or anticoagulative influence of free electrical charges is well shown in the experiments of Hardy on the action of the positively charged α-radiation from radium chloride upon globulin solutions. *Cf.* Journal of physiology, 1903, xxix, p. xxix of Proceedings of the Physiological Society, London.

one alone, even in the most favorable concentration. Thus with *Arenicola* and *Polygordius* larvæ, mixtures of sodium, magnesium, and calcium chlorides, in favorable proportions, will produce an almost normal medium, whereas magnesium chloride or calcium chloride, without the addition of the other, can effect only a partial counteraction of the toxicity of the pure sodium chloride.[1] It seems necessary to assume, provisionally at least, that each ion has a specific influence, as yet incompletely analyzed, upon the contractile tissues; also that the interaction of several is necessary for normal activity.

In the experiments about to be described, equivalent quantities of the different cations, usually in combination as chlorides, are added to the pure $\frac{m}{2}$ solutions of sodium chloride, sodium nitrate, or sodium acetate (the three chief salts employed). Evidence of antitoxic action, if such exists, is seen both in the greater duration of the ciliary movement, as compared with that in the pure solution, and in the greater force and frequency of the ciliary beats. When the action is distinct and pronounced, the organisms swim actively in the solution. Such active swimming is always seen in sodium chloride solutions to which small quantities of one of the following heavy metal salts have been added: manganese chloride, ferrous chloride, cobalt chloride, nickel chloride, cadmium chloride, zinc sulphate, lead chloride or acetate, cupric chloride. Certain ions sustain swimming movements far longer than others; with manganese chloride many larvæ may swim for fifteen minutes or more, while with cupric chloride, the least favorable of these salts, swimming usually ceases within fifteen or twenty seconds. Ciliary vibrations may continue long after their energy has become insufficient for swimming movements. In other cases, after the addition of certain ions, ciliary movement may be distinctly more active and prolonged than in the pure solution, yet from the first remain for the most part insufficient for swimming movements. This is the case with UO_2, whose antitoxic action is slight, also with Al, Cr, Fe''' (and the H-ion) by which ciliary activity may be considerably prolonged, though without ever becoming so energetic as with the more favorable bivalent ions. Where antitoxic action is slight, the effect is often indicated not so much by an increased duration of movement, as by a peculiar, quick, vibratory or trembling quality of movement quite different from that observed in the pure sodium-solution, which is slow and sluggish, and ceases usually within a few seconds.

[1] *Cf.* R. Lillie: *Loc. cit.*

TABLE I.

ACTION OF SOLUTION WITH LONGEST OBSERVED DURATION OF MOVEMENT.

Solution.	Result.
1a. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $FeCl_2$ . . .	Solution added 1.41½ P. M., Aug. 5. Active swimming for first few minutes. At 2.26 most cilia have ceased; a few larvæ show slow movement. 5.15: slow, regular ciliary beats[1] in a *few* larvæ; movement has almost ceased (3 h. 33 m.).
2a. $\frac{m}{2}$ NaCl + $\frac{m}{800}$ $FeCl_2$ . . .	1.45: Active swimming, longer continued than in 1a; some larvæ show slow regular ciliary beats at 7.21 P. M. (5 h. 36 m.).
3a. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ $FeCl_2$ . . .	1.49: Little swimming; cilia largely liquefy at once. A few movements at 2.11. No later movements seen (22 m.).
2a. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $CuCl_2$ . . .	2.16½: Active swimming at first; cilia have almost ceased after 20 m. (20 m.).
2b. $\frac{m}{2}$ NaCl + $\frac{m}{800}$ $CuCl_2$ . . .	2.27½: Active swimming at first, more favorable than 2a. Cilia active after 20 m. Have ceased after 60 m. (20 m.).
2c. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ $CuCl_2$ . . .	2.33: Swimming less active than in 2b; otherwise similar. No movement after 60 m. (20 m.).
3a. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $CoCl_2$ . . .	2.53: Active swimming, lasting 10 m. in some cases. Feeble ciliary movement in a few at 7.32 (4 h. 39 m.).
3b. $\frac{m}{2}$ NaCl + $\frac{m}{800}$ $CoCl_2$ . . .	2.57½: Swimming somewhat less active than 3a. No movement seen after 3.24 (26½ m.).
3c. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ $CoCl_2$ . . .	3.04: Little swimming; cilia largely cease and liquefy at once. 3.20: no movement perceptible.
4a. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $NiCl_2$ . . .	3.11: Active swimming at first. Movements cease relatively soon. No movement at 3.29 or later (18 m.).
4b. $\frac{m}{2}$ NaCl + $\frac{m}{800}$ $NiCl_2$ . . .	3.15: Swimming lasts longer than in 4a. Has mostly ceased at 4.09. A *few* movements seen at 7.48 (4 h. 33 m.).
4c. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ $NiCl_2$ . . .	3.47½: Little swimming; cilia soon cease and liquefy. Almost no movement at 3.59. No movement at 4.11 (1 h. 5 m.).
5a. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $CdCl_2$ . . .	3.53: Swimming is less active at first than with Co or Ni; cilia have largely ceased after 5 m. A few movements persist till 8.11 (4 h. 18 m.).
5b. $\frac{m}{2}$ NaCl + $\frac{m}{800}$ $CdCl_2$ . . .	4.02: Swimming is less active than with 5a. Most cilia cease in 5 m. A few remain active, as in 5a, till 8.16 (4h. 14 m.).
5c. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ $CdCl_2$ . . .	4.15: Swimming for first few minutes. Cilia cease soon; no movement after 4.20; cilia all liquefied at 4.50.
6a. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $ZnSO_4$ . . .	4.21: Active swimming at first; at 5.35 a few cilia active; at 6.10 no movement (1 h. 14 m.).

[1] Slow regular beating movements of individual cilia, with a rhythm of one stroke or less per second, and continuing often for fifteen minutes or more, are very characteristic of solutions containing ferrous salts like the above. This phenomenon is not seen with other salts, and seems dependent on some special peculiarity of the Fe''-ion.

Solution.	Result.
6b. $\frac{m}{2}$ NaCl + $\frac{m}{800}$ $ZnSO_4$ . . .	4.27: Cilia are less active than in 6a, and are largely liquefied at 4.30. A few continue active at 5.35. No movement at 6.10 (1 h. 8 m.).
6c. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ $ZnSO_4$ . . .	4.34½: Slow swimming at first; cilia are largely liquefied at 4.30. A few are feebly active at 5.37. No movement at 6.12 (1 h. 12½ m.).
7a. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $UO_2(NO_3)_2$.	Aug. 6, 3.34 P. M.: Rapid trembling ciliary movement at first, but only a few swim. After 4 m. cilia have largely ceased and liquefied. After 10 m. (3.44) only a few show movement; no movement seen later (10 m.).
7b. $\frac{m}{2}$ NaCl + $\frac{m}{800}$ $UO_2(NO_3)_2$.	3.41: Action like 7a. A few feeble ciliary and muscular movements continue at 3.54. No movement at 4.24 (13 m.).
7c. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ $UO_2(NO_3)_2$.	3.49½: Trembling movements, as in solutions 7a and 7b; a fair number show movement at 4.02. No movement at 4.28 (12 m.).
8a. $\frac{m}{2}$ NaCl + $\frac{m}{200}$ $MnCl_2$ . . .	3.54: Active swimming movements, heliotropic at first. This solution is very favorable, a few larvæ showing movement next morning (8.30 A. M.), after nearly 18 h. (18 h.).
8b. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $MnCl_2$ . . .	4.14: Like solution 8a, but no heliotropism; a few movements after 17 h. (17 h.).
8c. $\frac{m}{2}$ NaCl + $\frac{m}{800}$ $MnCl_2$ . . .	4.19: Less favorable than solution 8b. Movement has practically ceased after 6 h. (6 h.).
8d. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ $MnCl_2$ . . .	4.36½: Comparatively little movement in this solution; cilia have all ceased by 5.17 (40 m.).
9a. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $PbCl_2$ . . .	Aug. 20, 11.58 A. M.: Active swimming at first. Cilia remain active in a few at 2.40. No movement at 3.40 (1 h. 40 m.).
9b. $\frac{m}{2}$ NaCl + Ca $\frac{m}{800}$ $PbCl_2$* .	Aug. 10, 11.17: Larvæ swim at first. Weak ciliary movement persists in a fair number at 3.11. No movement observed later (*ca.* 4 h.).
9c. $\frac{m}{2}$ NaCl + Ca $\frac{m}{1600}$ $PbCl_2$ . .	Aug. 10, 11.26: Little swimming, and cilia cease soon; no movement at 11.32.

* The concentrations of these last two $PbCl_2$ solutions were inaccurately determined; they lay, however, almost certainly between the limits of $\frac{m}{800}-\frac{m}{1000}$ and $\frac{m}{1600}-\frac{m}{2000}$, respectively.

The comparative duration of movement in solutions containing equivalent quantities of different cations may be regarded as affording a certain approximate measure of their relative degree of antitoxic effectiveness. The following estimations are based upon this assumption.

The action of the alkaline earth cations may be conveniently summarized in a single paragraph. The action of heavy metal salts will then be described in greater detail.

It has already been shown that small quantities of calcium or magnesium chlorides will check or counteract the destructive action of pure

sodium chloride on cilia. Of the two, magnesium is the more effective.[1] Calcium and magnesium, as chloride or nitrate, act similarly in solutions of other sodium salts with monovalent anions (sodium nitrate, bromide, acetate); with sodium fluoride also a slight antitoxic action is observable; here, however, the magnesium ions in solution are insufficient for the production of any decided effect. With sodium sulphate also magnesium chloride acts antitoxically; larger quantities of magnesium are here required than in the case of salts with monovalent anions; calcium sulphate is too insoluble to permit any distinct antitoxic action with calcium salts. Similar statements hold true for sodium tartrate. The action of sodium citrate may also be partly antagonized by magnesium chloride; for the most favorable effect considerably larger quantities of magnesium are necessary than with sulphate or tartrate. Strontium and barium also counteract the toxicity of sodium chloride, nitrate, or acetate solutions. Of the two, strontium is the more favorable; it resembles calcium in being less immediately injurious to muscular contractility than magnesium. Barium is, on the whole, the most toxic of this group of cations; in low concentrations, however, it appears more favorable to ciliary movement than either calcium or strontium; on the other hand, it is rapidly destructive of muscular contractility, resembling in these two respects magnesium rather than calcium, while strontium exhibits a close similarity to calcium. These statements apply especially to solutions of $\frac{m}{2}$ NaCl, $NaNO_3$, and $NaCOOCH_3$, containing $BaCl_2$, etc., in $\frac{m}{400}$ concentrations. In higher concentrations the poisonous action of the barium partially obscures its antitoxic action, as in the case of many heavy metal cations.

Salts of the following bivalent heavy metal cations all exhibit antitoxic action to a greater or less degree: Mn, Fe'', Co, Ni, Cd, Zn, Pb, Cu, UO.

The following table gives the results of a number of experiments designed to determine the concentration in which the bivalent heavy metal ions exercise the most pronounced antitoxic action. The actual concentration of the heavy metal salt in the solution is given.[2] The figures in brackets in this and the following tables represent the

[1] At least in the case of ciliary movement; with muscular contraction the reverse is true.

[2] Thus 1 c.c. of $\frac{m}{2}$ $FeCl_2$, added to 99 c.c. $\frac{m}{2}$ NaCl, brings the concentration of $FeCl_2$ to $\frac{m}{200}$ without materially affecting the concentration of the sodium chloride.

longest observed duration of ciliary movement. Observation was made at frequent intervals, so that the times represent practically the maximal duration of movement in the respective solutions.

The most favorable concentration for the majority of the above bivalent cations thus appears to range from $\frac{m}{400}$ to $\frac{m}{800}$, $\frac{m}{1600}$ being, for the most part, distinctly too dilute. The more toxic ions, as Cu, Pb, are more effective in low concentrations, while with the less toxic, as Mn, the optimal concentrations are higher than the above. This will appear again below. The following table, summarizing a series of similar determinations with $\frac{m}{200}$ concentrations, shows less favorable conditions for most of the above cations.

TABLE II.

Solution.	Result.
1. $\frac{m}{2}$ NaCl + $\frac{m}{200}$ $FeCl_2$ . . .	Aug. 3, 4.03½: Active swimming at first; most cilia are in liquid droplets by 4.05, a few continue. At 4.34 a few show slow beating ciliary movements. 5.17: practically no movement. 6.17: no movement (1 h. 13½ m.).
2. $\frac{m}{2}$ NaCl + $\frac{m}{200}$ $CuCl_2$ . .	4.09½: Active swimming at first; at 4.15 almost all show movement. By 4.25 movement has almost ceased. No movement at 4.32 (15½ m.).
3. $\frac{m}{2}$ NaCl + $\frac{m}{200}$ $CoCl_2$ . .	4.19: Lively and rapid swimming movements; at 4.38 many are still swimming. 5.22: cilia have almost ceased. 6.18: feeble movement is seen in a single larva; no movement seen later (1 h. 59 m.).
4. $\frac{m}{2}$ NaCl + $\frac{m}{200}$ $NiCl_2$ . .	4.25: Swimming as in Solution 3, — somewhat less active. At 4.59 cilia are almost entirely liquefied and motionless; a few show feeble movements. 5.25: no movement (34 m.).
5. $\frac{m}{2}$ NaCl + $\frac{m}{200}$ $CdCl_2$ . .	4.44: Active swimming at first. By 5.00 most cilia are liquefied and inactive. A few feeble movements continue at 6.21 (1 h. 37 m.).
6. $\frac{m}{2}$ NaCl + $\frac{m}{200}$ $ZnSO_4$ . .	4.53½: Swimming is less active than in 5; cilia are largely liquefied and inactive by 4.55. A fair number show movement at 5.28; no movement at 6.25 (34½ m.)
7. $\frac{m}{2}$ NaCl + $\frac{m}{200}$ $UO_2(NO_3)_2$	5.03: Active trembling movements result at first, and a few swim: liquefaction follows quickly. No movement at 5.07.

Evidently $\frac{m}{200}$ is above the optimal concentration, except in the case of manganese (see below).

A distinct antitoxic action is thus evident with all of the above cations. If ranked in the order of their apparent favorability as estimated from the maximal duration of movement in the respective solutions, the order would be: Mn, Fe″, Co, Ni, Cd, Pb, Zn, Cu, UO_2, corresponding in a general way with the order of the numbers

representing their respective solution-tensions. Zn and Cd, however, are more toxic than their position in the scale would indicate. Experiments with other sodium salts (tabulated below) confirm in general the results of the preceding determinations, except that, as a rule, Fe'' is more toxic than appears above. But it is impossible to keep Fe'' from adding an additional charge and becoming Fe''' which is highly toxic, as will appear below. Freshly prepared solutions of ferrous chloride are much less toxic than those which have been allowed to stand for some time before using.

Action of Tetravalent and Trivalent Cations.

A number of experiments were tried with thorium nitrate, but with indecisive and almost negative results. The concentrations represented in Table III were employed. Only in the last two or three solutions was there any distinct evidence of antitoxic action; it will be seen from the table that the tetravalent ion does apparently exert a perceptible counteracting influence, but that this is slight and seen only in great dilution. In all concentrations above the two last, destructive action is marked, and cilia are arrested and liquefied almost immediately after addition of the solution.

TABLE III.

Solution.	Result.
1. $\frac{m}{2}$ NaCl + $\frac{m}{800}$ $Th(NO_3)_4$	Immediate liquefaction.
2. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ $Th(NO_3)_4$	" "
3. $\frac{m}{2}$ NaCl + $\frac{m}{3200}$ $Th(NO_3)_4$	" "
4. $\frac{m}{2}$ NaCl + $\frac{m}{6400}$ $Th(NO_3)_4$	" "
5. $\frac{m}{2}$ NaCl + $\frac{m}{12800}$ $Th(NO_3)_4$	" "
6. $\frac{m}{2}$ NaCl + $\frac{m}{25600}$ $Th(NO_3)_4$	Rapid liquefaction. Feeble movements continue for 2 m.
7. $\frac{m}{2}$ NaCl + $\frac{m}{51200}$ $Th(NO_3)_4$	Rapid liquefaction. Cilia continue 2–3 m.
8. $\frac{m}{2}$ NaCl + $\frac{m}{102400}$ $Th(NO_3)_4$	Rapid liquefaction in most cases. A few cilia show feeble movement after 20 m.
9. $\frac{m}{2}$ NaCl + $\frac{m}{204800}$ $Th(NO_3)_4$	Liquefaction. A few movments last for 4–5 m.

All three of the trivalent cations Al, Cr, and Fe''' act antitoxically. The order of favorability is that given. Fe''' has marked toxic properties and rapidly destroys cilia even at the most favorable concentra-

tions; Al and Cr are more favorable and, in suitable concentrations, may prolong movement considerably, though at best their efficiency is decidedly less than that of many bivalent cations.

Tables IV, V, and VI give the concentrations employed and the chief results gained.

TABLE IV.

Solution.	Result.
1. $\frac{n}{4}$ NaCl + $\frac{m}{800}$ $AlCl_3$ . . .	Aug. 6, 2.42: Rapid trembling movements at first; cilia liquefy and fuse at once, but movement of the partially fused and liquefied cilia continues for a short time. Movement ceases within 5 m.
2. $\frac{n}{4}$ NaCl + $\frac{m}{1600}$ $AlCl_3$ . . .	2.50½: Movement is more active than in 1, and a few swim. Slight movements continue till 3.29 (38½ m.).[1]
3. $\frac{n}{4}$ NaCl + $\frac{m}{3200}$ $AlCl_3$ . . .	2.58½: Active movement with swimming at first. At 4.08 only a few slight movements persist (70 m.).[2]
4. $\frac{n}{4}$ NaCl + $\frac{m}{6400}$ $AlCl_3$ . . .	3.05: Like Solution 3. A few slight movements persist at 4.07 (62 m.).
5. $\frac{n}{4}$ NaCl + $\frac{m}{12800}$ $AlCl_3$ . .	3.21½: Active movement, lasting longer than in Solution 4. A few movements continue at 5.55 (2 h. 33 m.).
6. $\frac{n}{4}$ NaCl + $\frac{m}{25600}$ $AlCl_3$ . .	Aug. 8, 3.01½: Active movement with swimming at first; a few feeble movements persist at 5.45 (2 h. 43½ m.).
7. $\frac{n}{4}$ NaCl + $\frac{m}{51200}$ $AlCl_3$ . .	3.07: Cilia are less active at first than in Solution 6. Slight movement is seen at 4.00; none at 4.30 (*ca*, 55 m.).
8. $\frac{n}{4}$ NaCl + $\frac{m}{102400}$ $AlCl_3$ . .	3.12½: Cilia only slightly active at first; a few continue active at 5.54 (2 h. 41½ m.).

[1] In another determination with this solution all cilia ceased within 20 m.

[2] Another determination showed only slight movement after 19 m.; no movement after 66 m.

In $\frac{m}{200}$ and $\frac{m}{400}$ concentrations, aluminium chloride does not prolong the activity of the cilia, which liquefy at once; although at first a rapid vibrating movement is seen, indicating a certain slight degree of favorable action. In these concentrations the toxic action of the salt preponderates to the exclusion of any distinct antitoxic effect. The same is true to still greater degree of chromium sulphate and ferric chloride.

Table V gives the results of a series of determinations with chromium sulphate.

The Cr-ion is thus less favorable than the Al-ion. Cilia rarely attain sufficient activity for swimming movements in these solutions

Table VI gives a similar series with ferric chloride.

TABLE V.

Solution.	Result.
1. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ $Cr_2(SO_4)_3$*	Aug. 7, 2.23: Active trembling movements at first, lasting *ca.* 2 m. Cilia soon liquefy and fuse.
2. $\frac{m}{2}$ NaCl + $\frac{m}{3200}$ $Cr_2(SO_4)_3$	2.29: Effect like Solution 1. Movement lasts a little longer, — from 4 to 5 m.
3. $\frac{m}{2}$ NaCl + $\frac{m}{6400}$ $Cr_2(SO_4)_3$	2.36: Active movement at first. A few slight movements are seen at 2.47 (11 m.).
4. $\frac{m}{2}$ NaCl + $\frac{m}{12800}$ $Cr_2(SO_4)_3$	2.41½: Active movement at first. A few movements last till 3.12 (20 to 25 m.).
5. $\frac{m}{2}$ NaCl + $\frac{m}{51200}$ $Cr_2(SO_4)_3$	Aug. 8, 2.36: Action more favorable than in Solution 4; a few movements last till 3.25 (45 m.).
6. $\frac{m}{2}$ NaCl + $\frac{m}{102400}$ $Cr_2(SO_4)_3$	2.43: Action like Solution 5. A few faint movements are seen at 3.53 (60 m.).
7. $\frac{m}{2}$ NaCl + $\frac{m}{204800}$ $Cr_2(SO_4)_3$	3.54½: Active trembling movement at first. Movement lasts distinctly longer than in Solutions 5 or 6. A few movements last till 4.27 (1 h. 32 m.).

* This is equivalent to $\frac{m}{800}$ $AlCl_3$, both concentrations being $\frac{n}{2400}$.

TABLE VI.

Solution.	Result.
1. $\frac{m}{2}$ NaCl + $\frac{m}{800}$ $FeCl_3$	Aug. 6, 3.13: Cilia fuse and liquefy almost at once; ciliary beats[1] last at most 30 sec.
2. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ $FeCl_3$	3.17: Cilia beat slowly for *ca.* 60 sec.; they soon fuse and liquefy.
3. $\frac{m}{2}$ NaCl + $\frac{m}{3200}$ $FeCl_3$	Aug. 7, 1.48: Action is like Solution 2; beating strokes of cilia continue for 1½ to 2 min.
4. $\frac{m}{2}$ NaCl + $\frac{m}{6400}$ $FeCl_3$	1.58: Movement is more active and long continued than in Solution 3. By 2.01 there is almost no movement, and cilia are fused.
5. $\frac{m}{2}$ NaCl + $\frac{m}{12800}$ $FeCl_3$	2.04: Active trembling movements at first; at 2.08 cilia are still active in a large proportion; many are slowly swimming. At 2.20 there is no movement, and cilia are liquefied.
6. $\frac{m}{2}$ NaCl + $\frac{m}{25600}$ $FeCl_3$	Aug. 8, 2.17: Movement continues longer than in Solution 5. At 2.32 cilia are active in a fair proportion. A *few* movements persist at 2.48 (31 m.).
7. $\frac{m}{2}$ NaCl + $\frac{m}{51200}$ $FeCl_3$	2.21: Most cilia cease soon; a few are active at 2.35, none at 2.49 (12½ m.).
8. $\frac{m}{2}$ NaCl + $\frac{m}{102400}$ $FeCl_3$	2.28: Movement is slight; a few cilia are active at 2.35; none at 2.50 (*ca.* 7 m.).

[1] The slow beating movements seen in solutions containing ferrous chloride also occur in solutions with ferric chloride, though to a much less degree.

The trivalent cations agree in exhibiting almost no antitoxic action in concentrations above $\frac{m}{1600}$, which is a favorable concentration for the majority of bivalent cations. The most pronounced action is obtained with highly dilute solutions ($\frac{m}{12800}$ to $\frac{m}{102400}$). On the whole $\frac{m}{25600}$ seems an approximate optimum with these cations, though marked effects are seen both with aluminium and chromium at four

TABLE VII — $\frac{m}{2}$ $NaCOOCH_3$.

Solution.	Result.
1. $\frac{m}{2}$ $NaCOOCH_3$	Aug. 18, 1.50 P. M.: Most cilia cease and liquefy at once. No trace of movement is seen at 1.57½.
2. $\frac{m}{2}$ $NaCOOCH_3 + \frac{m}{400}$ $MgCl_2$	1.54½: Cilia remain active with swimming movements; are still active at 7.20 P. M. A few larvæ show movement next morning after 18 h.
3. $\frac{m}{2}$ $NaCOOCH_3 + \frac{m}{400}$ $CaCl_2$	2.01½: Cilia largely cease within a few minutes; at 2.32 very little movement. No movement at 3.00. Muscular contractions continue some time longer (3.39).
4. $\frac{m}{2}$ $NaCOOCH_3 + \frac{m}{400}$ $SrCl_2$	2.09: Action as with Ca; by 2.35 cilia have all ceased; muscular contractions continue at 3.41.
5. $\frac{m}{2}$ $NaCOOCH_3 + \frac{m}{400}$ $BaCl_2$	2.15: Cilia remain active longer than in Solutions 3 and 4; 2.36: active ciliary movement in large proportion; no muscular contractions. A few cilia are active at 3.08; none at 3.43.
6. $\frac{m}{2}$ $NaCOOCH_3 + \frac{m}{400}$ $MnCl_2$	2.22: Active swimming at first; a large proportion show active ciliary movement at 4.44; a few cilia are active at 9.22. Movement had ceased next morning (7+ h.).
7. $\frac{m}{2}$ $NaCOOCH_3 + \frac{m}{400}$ $ZnSO_4$	2.26½: Swimming at first; at 4.48 cilia are active in large proportion. A few show movement at 8.22 P. M., and a trace of movement persists at 9.23 (7 h.).
8. $\frac{m}{2}$ $NaCOOCH_3 + \frac{m}{400}$ $CdCl_2$	2.43½: Active swimming at first; at 4.50 ciliary movement continues in many. A few movements persist at 7.29; none at 8.24 (4 h. 45 m.).
9. $\frac{m}{2}$ $NaCOOCH_3 + \frac{m}{400}$ $CoCl_2$	2.53½: Active swimming at first; cilia continue in a few larvæ at 9.27 P. M. No movement was found next morning (6 h. 35 m.).
10. $\frac{m}{2}$ $NaCOOCH_3 + \frac{m}{400}$ $NiCl_2$	2.58½: Swimming as in Solution 9. Ciliary activity as long continued, — large proportion show movement at 9.27 P. M. No movement next A. M. (6 h. 30 m.).
11. $\frac{m}{2}$ $NaCOOCH_3 + \frac{m}{400}$ $PbCl_2$	3.19: Active swimming at first. At 8.33 a fair proportion show movement; at 9.33 movement has almost ceased (6 h. 14 m.).
12. $\frac{m}{2}$ $NaCOOCH_3 + \frac{m}{400}$ $CuCl_2$	3.26: Cilia soon cease; a few show movement at 3.31. At 4.00 cilia are motionless and disintegrated.

times this dilution. On comparison with the most favorable concentration for the majority of bivalent heavy metals (*ca.* $\frac{m}{1600}$), the optimum is seen to range from sixteen to thirty-two times less. There is thus a great increase of antitoxic efficiency with increase of valence. Monovalent cations like potassium effect almost no counteraction of

sodium chloride solution, unless present in far higher proportions than are found necessary with bivalent ions,[1] while with the tetravalent cation thorium the only indications of antitoxic action were obtained at dilutions of $\frac{m}{102400}$ and $\frac{m}{204800}$. These determinations, therefore, afford a decided confirmation of the rule that the concentrations necessary for antitoxic action decrease very rapidly with increase in the valence of the active ion.

The following three series of determinations illustrate the power of bivalent cations to counteract the toxicity of pure solutions of other sodium salts, — acetate, nitrate, and sulphate. The bivalent metal salts are in $\frac{m}{400}$ concentration.

$\frac{m}{2}$ $NaCOOCH_3$ + $\frac{m}{400}$ $FeCl_2$ gave no result; a precipitate of ferrous acetate is formed.

It will be seen on comparison with the sodium chloride tables that sodium acetate is apparently less injurious than sodium chloride, since

TABLE VIII — $\frac{m}{2}$ $NaNO_3$.

Solution.	Result.
1. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $MgCl_2$	Active swimming at first; cilia remain active for two to three hours only.
2. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $CaCl_2$	No swimming; cilia cease within 10 m.
3. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $SrCl_2$	No swimming; cilia cease within 5 m.
4. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $BaCl_2$	No swimming; cilia remain active somewhat longer than in Solution 3.
5. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $MnCl_2$	Active swimming at first; cilia remain active for 2–3 h.
6. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $ZnSO_4$	Slow swimming at first; cilia remain active *ca.* 20 m.
7. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $CdCl_2$	Active swimming; cilia remain active for almost 2 h.
8. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $FeCl_2$	Swimming at first; cilia cease within a few minutes.
9. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $CoCl_2$	Active swimming at first; many show movement after 1 h. 30 m.; movement ceases in *ca.* 2 h.
10. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $NiCl_2$	Active swimming at first; movement in fair numbers after 1 h. 30 m.; ceases in *ca.* 2 h.
11. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $PbCl_2$	Swimming is less active than in Solution 9 or 10. A few movements are seen after 2 h.
12. $\frac{m}{2}$ $NaNO_3$ + $\frac{m}{400}$ $CuCl_2$	Slow swimming at first; cilia all cease and liquefy within 15 m.

in nearly all cases cilia remain active longer than in the corresponding chloride solutions. It is also noteworthy that in these concentrations

[1] *Cf.* R. LILLIE: *Loc. cit.*, 1901, p. 79. The H-ion is an exception to this rule. See below.

heavy metals are more effective than the alkaline earth metals. Mn remains the most effective of the bivalent heavy metal cations, as before, with Co, Ni, Zn, Pb, Cd, and Cu, following in the order named.

With sodium nitrate, similar results appear. This salt, however, is decidedly more toxic than the acetate, ciliary movement ceasing in all cases much sooner than in the corresponding acetate solutions.

The same salts were used with $\frac{m}{2}$ NaCl. The movement lasts longer than with $\frac{m}{2}$ $NaNO_3$, but not as long as with $\frac{m}{2}$ $NaCOOCH_3$.

TABLE IX — $\frac{m}{2}$ NaCl.

Solution.	Result.
1. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $MgCl_2$	Active swimming at first; cilia remain active more than 7 h.: have ceased after 9 h.
2. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $CaCl_2$	Cilia liquefy and cease very soon; no movement after 5 m. Muscular contractions continue for 2 h.; have ceased after 3 h.
3. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $SrCl_2$	Action like Solution 2. Muscular contractions cease sooner than with $CaCl_2$ (within 1 h.).
4. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $BaCl_2$	Cilia continue somewhat longer than with Ca or Sr. Muscular contractions cease within 5 m.
5. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $MnCl_2$	Active swimming at first; cilia continue in a few for more than 6 h.
6. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $ZnSO_4$	Slow swimming at first; cilia have almost ceased after 1 h. 30 m.
7. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $CdCl_2$	Swimming at first; cilia remain feebly active after 5 h.
8. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $FeCl_2$	Swimming at first; a few cilia remain active more than 3 h.; all have ceased in 4 h.
9. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $CoCl_2$	Swimming at first; movement lasts for 3 to 4 h., as in 8.
10. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $NiCl_2$	Action similar to Solution 9 (3–4 h.).
11. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $PbCl_2$	Swimming at first; a few movements continue for more than 3 h.
12. $\frac{m}{2}$ NaCl + $\frac{m}{400}$ $CuCl_2$	Slow swimming at first; a few cilia are slowly active after 25 m.; no movement after 50 min.

The next table gives the results of a similar series with sodium sulphate. The antitoxic action here is relatively slight, since a higher concentration than $\frac{m}{400}$ is required to counteract most effectively the toxic action of salts with bivalent anions.

The above results are given *in extenso* chiefly to illustrate two facts: first, that the different cations exhibit highly varying antitoxic efficiency, and second, that the order of efficiency remains in general the same whatever the sodium salt employed. The action, therefore, depends in each case on some property specific to the particular cation employed. *All* of the above cations exhibit more or less antitoxic action, mercury being the only bivalent heavy metal that gives

uniformly negative results. It was found that the addition of mercuric chloride in all concentrations ranging from $\frac{m}{200}$ to $\frac{m}{204800}$ failed absolutely to counteract the toxicity of pure $\frac{m}{2}$ NaCl to any appreciable degree. Apparently, the specific toxicity of this cation completely overbalances whatever antitoxic influence its positive charges may exercise.

It is hardly possible, using the above data, to form any exact numerical estimate of the relative antitoxic efficiency of the above

TABLE X — $\frac{m}{2}$ Na_2SO_4.

Solution.	Result.
1. $\frac{m}{2}$ Na_2SO_4	Cilia have completely ceased after 3 or 4 m.
2. $\frac{m}{2}$ Na_3SO_4 + $\frac{m}{400}$ $MgCl_2$	Swimming at first; most cilia cease within 60 m. No movement after 2 h.
3. $\frac{m}{2}$ Na_2SO_4 + $\frac{m}{400}$ $CaCl_2$	Cilia cease and liquefy at once. No movement after 2 m.
4. $\frac{m}{2}$ Na_2SO_4 + $\frac{m}{400}$ $MnCl_2$	Swimming at first; almost all cilia cease within 60 m. No movement after 1 h. 30 m.
5. $\frac{m}{2}$ Na_2SO_4 + $\frac{m}{400}$ $ZnSO_4$	Slow swimming at first; cilia remain active 10–15 m.; all cease within 23 m.
6. $\frac{m}{2}$ Na_2SO_4 + $\frac{m}{400}$ $CdCl_2$	Swimming at first; movement lasts longer than in Solution 5; slight activity after 40 m.; none after 75 m.
7. $\frac{m}{2}$ Na_2SO_4 + $\frac{m}{400}$ $FeCl_2$	Slight swimming; after 27 m. a few slow ciliary beats persist; no movement after 66 m.
8. $\frac{m}{2}$ Na_2SO_4 + $\frac{m}{400}$ $CoCl_2$	Slow swimming; movement has practically ceased after 60 m.
9. $\frac{m}{2}$ Na_2SO_4 + $\frac{m}{400}$ $NiCl_2$	Action like Co; slight movement after 25 m.; none after 60 m.
10. $\frac{m}{2}$ Na_2SO_4 + $\frac{m}{400}$ $PbCl_2$	Cilia cease soon; little movement after 3 m.; none after 30 m.
11. $\frac{m}{2}$ Na_2SO_4 + $\frac{m}{400}$ $CuCl_2$	Slight swimming at first; cilia have almost ceased in 5 m.; no movement after 10 m.

cations. It seems admissible, however, to use the average maximal duration of ciliary movement in the several solutions containing any particular cation as a certain index of the antitoxic efficiency of that cation, and so to range the different cations in their order of efficiency. The following table summarizes the above four series of determinations. Under the symbol of each cation is given the longest observed duration of ciliary movement with each of the four salts. The last line gives the average of the four (or three) determinations for each cation:

The order of effectiveness in the case of the bivalent heavy metal cations then runs somewhat as follows: Mg, Mn, Co, Cd, and Ni (which are very similar in their action), followed by Pb, Zn, Fe″, Cu.

Mg and Mn are remarkably similar in their action; so also are Co and Ni; Cd is somewhat more toxic than corresponds to its position in the solution-tension scale, and is very similar to Co and Ni. Both Fe″ and Zn appear also more toxic than can be explained on the solution-

TABLE XI.

	Mg	Ca	Sr	Ba	Mn	Zn	Cd	Fe″	Co	Ni	Pb	Cu
	h. m.	h. m.	h. m.	h. m.	h. m.	h. m.	h. m.	h. m.	h. m.	h. m.	h. m.	h. m.
$NaCOOCH_3$	18 00	0 30	0 20	0 50	7 00	7 00	4 45		6 30	6 30	6 00	0 10
$NaNO_3$	3 00	0 10	0 05	0 05	2 30	0 20	2 00	0 05	2 00	2 00	2 30	0 15
NaCl	9 00	0 05	0 05	0 06	6 00	1 30	5 00	3 30	3 30	3 30	3 00	0 30
Na_2SO_4	1 00	0 02			1 00	0 15	0 40	0 30	0 30	0 30	0 15	0 05
Av. maximal duration of movement.	7 45	0 12	0 10	0 20	4 07	2 16	3 06	1 20	3 08	3 00	2 56	0 15

tension hypothesis; in the case of Fe″ this is probably due in large part to admixture with Fe‴-ions. Pb is only slightly more toxic than Ni; while Cu, with a decidedly lower solution-tension, is far more toxic than Pb. Hg, as has been seen, is by far the most toxic of the bivalent heavy metals; this is in accordance with the low value of its solution-tension.

Such figures furnish only a rough indication of the relative antitoxic efficiency of the ions, or of their toxicity, — which may be regarded as the reciprocal of the antitoxic efficiency. If we estimate the average effectiveness of each cation from the average maximal duration of movement for the three concentrations used in the series of Table I, a similar result appears.

TABLE XII.

	Mn	Zn	Cd	Fe″	Co	Ni	Cu	(UO_2)	Pb
	h. m.	h. m.	h. m.	h. m.	h. m.	h. m.	h. m.	h. m.	h. m.
	17 00	1 15	4 15	3 30	4 40	0 15	0 20	0 10	1 40
	6 00	1 06	4 15	5 30	0 30	4 30	0 20	0 13	4 00
	0 40	1 12	05	0 20	0 15	0 15	0 15	0 12	0 0
Av.	8 00	1 10	2 50	3 06	1 48	1 40	0 18	0 12	1 50

According to these averages, the order of toxicity (Mn, Fe″, Cd, Co, Pb, Ni, Zn, Cu, UO_2) diverges somewhat less from the theoretical (on the solution-tension hypothesis), though Zn is still decidedly out of place. The agreement is thus of a general kind only, and although any indication of a possible connection between toxicity and solution-tension must be regarded as of great interest, it seems necessary to conclude that this latter property is only one of a number of variables, each of which plays a part in determining the physiological action of the ion. Further indications of the relative toxicity of several of the above ions may be obtained by testing the direct action of the pure $\frac{m}{3}$ or $\frac{m}{4}$ solution of the salt (Table XIII).

TABLE XIII.

Solution.	Result.
1. $\frac{m}{2}$ $MnCl_2$	Larvæ swim actively at first; ciliary movement lasts *ca.* $1\frac{1}{2}$ h.
2. $\frac{m}{3}$ and $\frac{m}{4}$ $MnCl_2$	Similar results.
3. $\frac{m}{3}$ $ZnSO_4$	Destroys movement at once.
4. $\frac{m}{2}$ and $\frac{m}{3}$ $CdGl_2$	Larvæ swim actively at first; movement ceases in 6 or 7 m.
5. $\frac{m}{2}$ and $\frac{m}{3}$ $CoCl_2$	Active swimming at first; cilia remain active *ca.* 20 m.
6. $\frac{m}{2}$ and $\frac{m}{3}$ $NiCl_2$	Action like $NiCl_2$; ciliary movement lasts 15–20 m.
7. $\frac{m}{3}$ $FeCl_2$ (freshly prepared)	Movement ceases at once and cilia fuse.
8. $\frac{m}{3}$ and $\frac{m}{4}$ $CuCl_2$	Cilia cease in 15 secs.
9. $\frac{m}{3}$ $Pb(COOCH_3)_2$	Movement ceases at once.

Pure solutions of $\frac{m}{2}$ $MnCl_2$ are surprisingly non-toxic, comparing favorably with $\frac{m}{2}$ $MgCl_2$ solutions in this respect. Cobalt and nickel chlorides, and to a less degree cadmium chloride, also sustain movement for some little time. In the other solutions cilia are quickly destroyed. Little weight is to be laid on these determinations, but it is interesting to note that pure solutions of heavy metal chlorides, provided the solution-tension of the cation is relatively high (Mn, Cd, Co, Ni), can support ciliary activity, if only for a short time. The action of the pure salt is here complicated by the acidity of the solutions.

Antitoxic Action of Hydrogen Ions.

In moderate concentrations acids inhibit the majority of physiological processes, including ciliary movement. It is interesting to observe, however, that in very dilute concentrations the H-ion may act antitoxically like any other cation. The following determinations indicate this:

TABLE XIV.

Solution.	Result.
1. $\frac{m}{2}$ NaCl + $\frac{m}{200}$ HCl	Ciliary and muscular movement cease at once.
2 $\frac{m}{2}$ NaCl + $\frac{m}{400}$ HCl	Movement ceases somewhat less promptly than in Solution 1.
3. $\frac{m}{2}$ NaCl + $\frac{m}{800}$ HCl	Cilia remain active *ca.* 30 sec.; all cease within 2 m.
4. $\frac{m}{2}$ NaCl + $\frac{m}{1600}$ HCl	Ciliary movement lasts 30–60 secs.; ceases within 2 m.
5. $\frac{m}{2}$ NaCl + $\frac{m}{3200}$ HCl	Movement continues longer than in Solution 4. Ceases in 3–4 m.
6. $\frac{m}{2}$ NaCl + $\frac{m}{6400}$ HCl	Movement continues much longer than in Solution 5; it persists in many after 1 h.; in a few after $1\frac{1}{2}$ hrs.
7. $\frac{m}{2}$ NaCl + $\frac{m}{12800}$ HCl	Movement is active at first, with swimming in some cases; a few slight movements persist after 1 h. 48 m.
8. $\frac{m}{2}$ NaCl + $\frac{m}{25600}$ HCl	Most cilia cease within 2 m.; only a few slight movements remain after 15 m.

HCl seems thus most favorable in concentrations of $\frac{m}{6400}-\frac{m}{12800}$; in these solutions movements may be sustained for $1\frac{1}{2}$ to 2 hours. Similar results were obtained with $\frac{m}{2}$ $NaNO_3$. Here also movement begins to be slightly prolonged at $\frac{m}{3200}$ concentrations; at $\frac{m}{6400}$ movement persisted after 55 m., but ceased after 85 m.; at $\frac{m}{12800}$ movement lasted for 30 to 45 minutes. Movement does not last so long as with sodium chloride, in accordance with the greater specific toxicity of the nitrate ion. (Compare Tables VIII and IX.)

The H-ion is remarkable among monovalent cations in exhibiting marked antitoxic action at the above dilutions, which are quite ineffective with the alkali metals and ammonium, or even with bivalent metals.

Probably the high migration-velocity of the H-ion is partly responsible for its exceptional antitoxic efficiency, which resembles that of a trivalent rather than of monovalent cation, it being evident that the sphere of influence of an ionic charge must increase directly with the speed of the ion bearing it. Again, the solution-tension of the H-ion is low, being less than that of Pb, and approaching that of Cu;

this peculiarity must also be considered in attempting to account for its physiological action. It is to be expected that such a combination of characteristics will have the effect of greatly intensifying the physiological activity of an ion.

Relation of the Antitoxic Action of Cations to the Valence of the Anion of the Toxic Salt.

It was observed by Loeb[1] that in order to counteract the toxicity of sodium salts with bivalent anions, larger quantities of the antagonizing cation were necessary than if the anion was monovalent; if the latter was trivalent, still larger quantities of the cation were required. He inferred that in general the toxicity of a salt increases with the valence of its anion; hence the need of increasingly large quantities of a given cation to antagonize salts with bi- and trivalent anions; he estimated that the equivalent antitoxic doses of calcium chloride, with solutions of sodium acetate, sulphate, and citrate, respectively, stood in approximately the proportion of 1 : 4 : 16.

A similar relation appears to hold true for ciliary movement. In the following series (Table XV) $MnCl_2$ was used to antagonize $\frac{m}{2}$ solutions of sodium acetate, sodium sulphate, and sodium citrate, respectively. $MnCl_2$ was present in each solution in concentrations ranging from $\frac{m}{50}-\frac{m}{800}$.

TABLE XV.

Action of Solution.

Concentration of $MnCl_2$	Na_3-citrate	Na_2SO_4	$NaCOOCH_3$
$\frac{m}{50}$	Movement lasts for more than 1 h. 45 m.	Movement for 7 h. 30 m.	Movement for more than 7 h.
$\frac{m}{100}$	Movement for 49+ m.	Movement for 4 h.	Movement for 7+ h.
$\frac{m}{200}$	Movement has almost ceased after 12 m.; none after 50 m.	Movement for 2 h.	Movement for 6–7 h.
$\frac{m}{400}$	No movement after 12 m.	Movement ceases within 1 h.	Movement for 6–7 h.
$\frac{m}{800}$	Cilia all cease within 10 m.	Ceases within an hour.	Movement for 6–7 h.

It is seen that the citrate is not distinctly antagonized until the concentration of $MnCl_2$ reaches $\frac{m}{50}-\frac{m}{100}$. With sulphate a decided

[1] Loeb, J.: This journal, 1902, vi, p. 429.

action begins with $\frac{m}{200}$, although $\frac{m}{400}$ is also favorable, while with acetate a marked action is evident at concentrations of $\frac{m}{800}$.

Another series with cobalt chloride, using sodium citrate, tartrate, sulphate, and acetate, respectively, yielded a similar result.

TABLE XVI.

ACTION OF SOLUTION.

Concentration of $CoCl_2$.	Na_3-citrate.	Na_2-tartrate.	Na_2SO_4.	$NaCOOCH_3$.
$\frac{m}{50}$	Movement ceases within 10 m.; a few larvæ swim at first.	Movement seen aft. 41 m.; ceases within the hour.	Movement in good many after $5\frac{1}{2}$ h.	Movement after $4\frac{3}{4}$ h.
$\frac{m}{100}$	Movement ceases within 10 m.	Movement lasts Ca 30 m.	Movement has almost ceased in 4 h.	Similar to above.
$\frac{m}{200}$	Movement ceases within 10 m.	Movement lasts Ca 30 m.	Less favorable than above; a few movements last 4 h.	More favorable than $\frac{m}{100}$.
$\frac{m}{400}$	Movement ceases almost immediately.	Movement ceases within 15 m.	Movement ceases mostly within 15 m.; faint movement after 60 m.	Still more favorable; a few movements after 9 h.
$\frac{m}{800}$	Movement ceases almost immediately.	Ceases in 12–13 m.	Similar to $\frac{m}{400}$.	Movement lasts more than nine hours.

Thus $CoCl_2$, even at $\frac{m}{50}$ concentration, can antagonize citrate only very slightly. With sulphate and tartrate no decided action is seen with increasing concentrations of the antagonizing salt until $\frac{m}{200}$ is reached, while $\frac{m}{800}$ and $\frac{m}{400}$ concentrations of $CoCl_2$ give the most favorable action with acetate. Similarly nickel chloride was found to produce a marked antitoxic effect with acetate at dilution of $\frac{m}{800}$ and $\frac{m}{400}$; with sulphate, a comparable action was seen only at $\frac{m}{50}$ and $\frac{m}{100}$. The toxicity of the citrate is only slightly counteracted by the ions Co and Ni; the best effects appeared with so high concentrations as $\frac{m}{10}$ to $\frac{m}{30}$. The relatively high specific toxicity of these cations prevents their efficiency at such concentrations from approaching that of Mn; movement could not be prolonged for more than 15 minutes at best with either $NiCl_2$ or $CoCl_2$.

A number of similar determinations were made with the relatively non-toxic cations Mg and Mn. Mixtures of Na_3-citrate and $MnCl_2$ containing $\frac{m}{50}$ $MnCl_2$ were found to prolong movement for periods of

one to two hours, as seen in Table XV; the same was observed with concentrations of $\frac{m}{30}$, $\frac{m}{20}$, and $\frac{m}{10}$. In the last two solutions (mixtures of 9 and 4 volumes $\frac{m}{2}$ Na_3-citrate with 1 volume $\frac{m}{2}$ $MnCl_2$ respectively) the best results appeared; swimming movements continued for ten minutes, and slow ciliary movement persisted for considerably more than an hour. At best, activity could not be maintained for more than an hour and a half to two hours.

With $MgCl_2$ two series of determinations were made, using the following mixtures:

(1)	9 volumes $\frac{m}{2}$	Na_3-citrate	+ 1 volume $\frac{m}{2}$	$MgCl_2$,		
(2)	8 "	"	+ 2 "	"	,etc.	
(9)	1 "	"	+ 9 "	"		

The most favorable action appeared in the mixtures of 7 volumes Na-citrate + 3 $MgCl_2$, and 8 volumes citrate + 2 $MgCl_2$ ($\frac{m}{6.6}$ and $\frac{m}{10}$ $MgCl_2$, respectively); in the former solution, movement continued for more than 6 hours in the one case and for approximately 4 hours in the other. The comparatively great toxicity of the citrate ion, as compared with that of the other anions used above, is seen in the fact that under no conditions as yet ascertained could movement be prolonged for periods comparable to these possible with NaCl, $NaCOOCH_3$, or Na_2SO_4.

Accurate determinations of the comparative toxicity of the various anions is a subject for future investigation. It is probable that as in the case of the cations certain variables other than valence — possibly, as Mathews holds, the fixation-intensity of the ionic charges — will eventually be shown to play an important rôle.

Summary.

1. Pure solutions of sodium salts act destructively on cilia, producing liquefaction and cessation of movement. Potassium and ammonium salts, on the contrary, permit movement to continue for some time.

2. This destructive action of sodium salts is not prevented by the addition of other anions to the solution.

3. The majority of cations exhibit antitoxic action, *i. e.*, prevent liquefaction and enable movement to continue. Antitoxic action appears thus to be a function of the electrical positivity of the cation.

4. The antitoxic efficiency of the cation varies with its valence, trivalent ions (Al, Cr, Fe‴) exhibiting their most favorable action at concentration from 16 to 32 times less than those found necessary for most bivalent cations. The order of efficiency is Al, Cr, Fe‴. Monovalent ions (except H) require higher concentrations than bivalent ions for the production of antitoxic effects.

5. The heavy metal cations exhibit varying antitoxic efficiency; in general, this is greater with metals of high solution-tension. The order of antitoxic efficiency corresponds somewhat closely with the order of the metals in the solution-tension scale, indicating that a relation exists between the physiological activity of an ion and the "fixation-intensity" of its charge or charges.

6. In dilutions of $\frac{m}{6400}-\frac{m}{12800}$ the H-ion exhibits well-marked antitoxic action.

7. The quantity of a given cation required to counteract the toxicity of a salt is found to increase rapidly with an increase in the valence of the anion of that salt.

THE RELATION BETWEEN THE DECOMPOSITION-TENSION OF SALTS AND THEIR ANTI-FERMENTATIVE PROPERTIES.

By HUGH McGUIGAN.

[*From the Hull Physiological Laboratories, University of Chicago.*]

IT is well known that sufficiently large doses of acids, salts, or bases will inhibit the action of ferments. Exact quantitative determinations of the minimum amount required to prevent the action of any specific ferment have not been made, except for a few acids and bases, and a very few salts; nor has it been determined how the salts act. No correlation between the antifermentative and the physical properties of the elements has been discovered.

It was the object of this research to determine the minimum dose of the various salts which will just prevent the action of malt diastase, and to determine, if possible, how far solution-tension, atomic weight, and other physical properties enter into the action. It was hoped that if it could be discovered how salts prevent fermentation, something might be learned of the nature of the ferment action.

Mathews[1] has recently shown that the poisonous action of any element depends very largely on its solution-tension, or, more properly, upon the affinity of the atom for its electrical charge. He found that it was possible to calculate approximately the minimum fatal dose of a salt from its solution-tension. He has also shown that the solution-tension of any ion determines its physiological action, since the inhibiting action of positive ions upon motor nerves, or the stimulating action of the negative ions, is inversely proportional to the solution-tension of the ion[2] (Haftintensität).

In this present work I sought to discover whether the same factors prevailed in the antifermentative action of salts, and, if so, to secure if possible more accurate quantitative data than Dr. Mathews was able to secure from his study of the minimum fatal dose for the Fundulus eggs. If exact data can be secured for a number of ferments, and for

[1] MATHEWS: This journal, 1904, x, p. 290.

[2] MATHEWS: Science, 1903, p. 729.

a number of different kinds of cells, we believe that from this data, and from the physical properties of a salt, it will be possible to predict its physiological action with even greater accuracy than the properties of a missing element in the periodic system.

The minimum fatal dose of salts will undoubtedly differ, slightly at least, with the kind of ferment. For example, Cole[1] finds that most salts, even in minute amounts, depress the action of invertin, while pectase[2] acts only in presence of small amounts, and amylolytic ferments in general are stimulated by small amounts of salts. It is also clear that in some cases the accelerator of one diastase may be the paralyzer of another.[3] Alkalies, even in the minutest amount, inhibit the action of diastase and many other ferments; while Martin and Effront[4] found that alkalies, in small doses favor and acids retard the action of œnoxidase. An alkaline reaction is necessary for the action of trypsin. Effront[5] found that 0.005 gram sodium carbonate, per 100 c.c., diminished diastase power almost 20 per cent; Langley[6] found that 0.0015 gram of the same salt causes a retardation of the action of ptyalin. I find that NaOH $\frac{n}{1538}$ and KOH $\frac{n}{1666}$ stop the action completely.

While a slight degree of acidity favors the action of diastase, a greater acidity inhibits it. Kjeldahl,[7] working with a dextrin solution, found the activity maximum when the acidity of the medium was $\frac{n}{2450}$, temperature 59°, time twenty minutes. Five times this strength, or $\frac{n}{490}$ caused almost complete inhibition. Effront, working with an infusion of filtered malt, obtained the same results for the acidity necessary for maximum action, but found only a small inhibition (2 per cent) with $\frac{n}{490}$, — the strength which Kjeldahl found to inhibit. This discrepancy was possibly due to the presence of bases in Effront's malt infusion. Maximum activity was found with both acids as follows:

	H_2SO_4	HCl
Kjeldahl	$\frac{n}{2450}$	....
Effront	$\frac{n}{2400}$	$\frac{n}{1216}$

I find that $\frac{n}{990}$ and $\frac{n}{1000}$ completely inhibits action.

[1] Cole: Journal of physiology, 1903, xxx, p. 283.
[2] Effront: Les enzymes, p. 290.
[3] Duclaux: Traité de biologie, ii, p. 373.
[4] Effront: Les enzymes, p. 358.
[5] Effront: Les enzymes, p. 325, etc.
[6] Langley: Journal of physiology, 1883, iv, p. 18.
[7] Kjeldahl: Les enzymes, p. 135.

Grützner[1] found that small amounts of all acids increased the action of pancreatic amylopsin. The optimum for hydrochloric acid which he gives is almost identical with that found by Kjeldahl with H_2SO_4 for diastase. These results correspond to those of Kübel[2] and Vernon.[3] Cole[4] finds that the action of ptyalin on starch is increased by the addition of very small amounts of acids, and also by neutral salts of strong monobasic acids; and that the action is decreased by the action of larger amounts of acids and by the addition of weak monobasic, dibasic, and tribasic acids.

The present work has been directed only toward the complete inhibition of diastase by salts.

Methods.

1. A starch paste was prepared by boiling one gram of starch with water sufficient to make the final volume 100 c.c.

2. The diastase solution (5 per cent) was made from commercial diastase (Diastas-Weyeth), and purified so that it gave no reduction with Fehling's solution. It was used when freshly prepared, — in no case more than five hours old.

3. Salt solutions of known strengths were made from chemically pure preparations, with the usual precautions.

The work was carried on in medium-sized test-tubes. A known quantity of the electrolytes, 2 c.c. of the starch solution and 0.8 c.c. of the diastase solution, and water sufficient to bring the final volume to 10 c.c., was added to each tube. The order in which these were added was: electrolyte, water, starch, and diastase. In each case the test-tube was shaken before and after the addition of the ferment. The final volume contained 0.4 per cent starch, and 0.04 per cent diastase. The action was allowed to continue sixty minutes at 40° C. A control consisting of starch and diastase without the electrolyte was made with each experiment. At the end of sixty minutes the contents of the tubes were boiled to stop the action of the ferment, and after removal of the salt, where this was necessary, were tested for sugar by the ordinary Fehling's test.

[1] Grützner: Archiv für die gesammte Physiologie, 1902, xci, p. 195.

[2] Kübel: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 276.

[3] Vernon: Journal of physiology, 1901, xxvii, p. 174.

[4] Cole: *Ibid.*, 1903, xxx, p. 202.

Table I shows the concentration of each salt just sufficient to prevent the formation of sugar in one hour at 40° C.

TABLE I.

$AgNO_3$	$< \frac{n}{100000}$	$CdCl_2$	$\frac{n}{143}$	KI		n
Ag_2SO_4	$< \frac{n}{100000}$	$ZnCl_2$	$\frac{n}{69}$	NaCl	$>$	$3n$
$AuCl_3$	$\frac{n}{33333}$	$MnCl_2$	$\frac{4n}{25}$	KCL	$>$	$3.5n$
$HgCl_2$	$\frac{n}{30000}$	$AlCl_3$	$\frac{3n}{10}$	KOH		$\frac{n}{1666}$
$CuCl_2$	$\frac{n}{8333}$	$MgCl_2$	$\frac{n}{1}$	NaOH		$\frac{n}{1538}$
$PbNO_3$	$\frac{n}{30}$(?)	LiCl	$1.4n$	HCl		$\frac{n}{990}$
HCl	$\frac{n}{990}$	$CaCl_2$	$\frac{n}{4}$	H_2SO_4		$\frac{n}{1000}$
$NiCl_2$	$\frac{n}{910}$	$SrCl_2$	$\frac{2n}{5}$	$KHSO_4$		$\frac{n}{500}$
$CoCl_2$	$\frac{n}{100}$	$BaCl_2$	$\frac{9n}{10}$	$(COOH)_2$		$\frac{n}{714}$
$FeCl_3$	$\frac{n}{333}$	NaI	$\frac{3n}{5}$	$H \cdot C_2H_3O_2$		$\frac{4n}{25}$

The concentrations given for NaCl and KCl — $3n$ and $3.5n$ respectively do not stop the formation of sugar completely, but the amount formed is very small. A greater concentration could not be used with any degree of accuracy, and for this reason the figures are given. They are approximately correct.

If the poisonous strengths given in Table I are compared with the solution-tensions of the same elements given in Table II, or with the heat of ionization,[1] it is seen that there is a remarkable resemblance between them. Thus, potassium, sodium, and barium salts are the least inhibitive, and have the highest solution-tensions. Silver, gold, mercury, copper, and the acids, with low solution-tensions, inhibit very strongly.

In the results obtained by me, the most marked exceptions from the solution-tensions are the salts of cobalt and lead. Mathews[2] found cobalt to be exceptionally weak in its action on Fundulus, and I find it an exception in its action on diastase. While the conditions of the experiments were alike so far as they could be made, no good explanation can be given at present for such marked exceptions. The salts of lead are known to be relatively strong poisons, and, for this reason, the exception was a surprise. It is possible that the lead united with the starch, and was thus removed from solution. Further work, particularly with ferments of less vitality than malt diastase, will, I think, clear up these discrepancies.

[1] OSTWALD: Zeitschrift für physikalische Chemie, 1903, xi, p. 507.

[2] MATHEWS: This journal, *loc cit.*

The relation between the solution-tension and the antifermentative action of salts can be graphically represented by plotting (Fig. 1). If we use the atomic weights as abscissæ, and the poisonous doses given in Table I as ordinates, we obtain a curve strikingly parallel

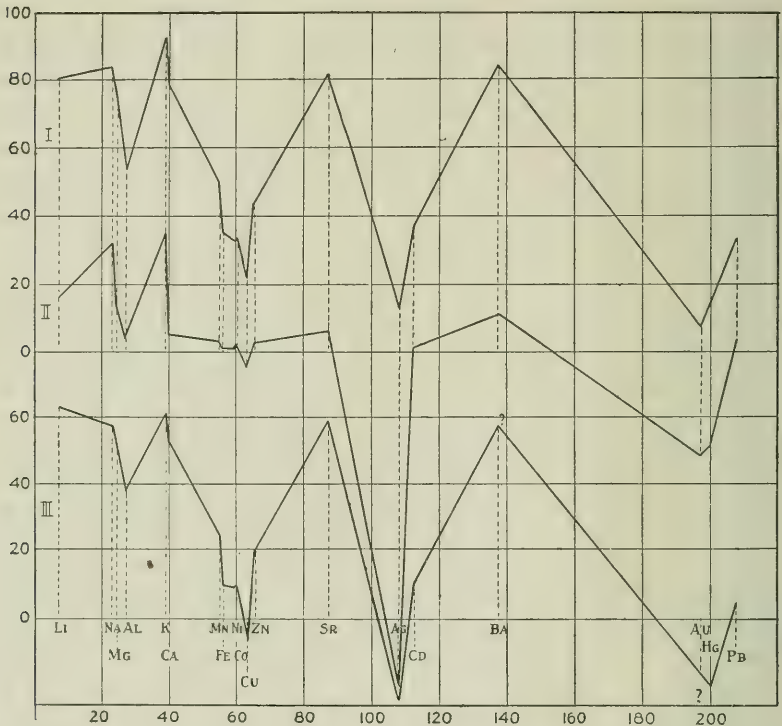

FIGURE 1.—I. Tension curve. The abscissæ represent atomic weights; the ordinates represent decomposition-tension in volts; two divisions equal one-tenth volt.

II. Poison curve. The abscissæ represent atomic weights; the ordinates show the relative strengths of the chlorides of the metals necessary to prevent the action of the ferment. The figures are obtained from Table I by multiplying by 100; then one division above the line equals ten times the normal strength; one division below the line equals one-tenth normal.

III. Heat of ionization. The abscissæ represent atomic weights. The ordinates represent calories; one division equals one large calorie. The values above the line are positive; those below are negative.

with that obtained by using the same abscissæ and the solution-tensions given in Table II as ordinates. The general relationship is so close that we are justified in stating that the antifermentative action of a salt varies inversely with its decomposition-tension. Furthermore

both ions are concerned in the process. For example, NaI is more poisonous than KI. The solution-tension of the former is lower than that of the latter. For a similar reason, both of these are much more poisonous than the chlorides of the same metals. The concentrations given for the hydroxides may be considered as of the same strength, as in this work we do not believe the limit of error will enable us to state exactly a difference between say a $\frac{n}{990}$ and a $\frac{n}{1000}$, or even a wider range, in the case of acids or alkalies. It is clear, therefore, that the poisonous or inhibiting action is inversely proportional to the solution-tension of each ion, and varies inversely with the decomposition-tension of the salt, or the sum of the solution-tensions of the ions.

It is remarkable that the hydrates are more poisonous than the acids, whereas for most forms of protoplasm the reverse is the case.

TABLE II.

SOLUTION-TENSIONS.

K	+ 2.92	Mn	+ 0.798	Hg	− 1.027
Na	+ 2.54	Zn	+ 0.493	Ag	− 1.048
Ba	+ 2.54	Cd	+ 0.143	Au	< − 1.356
Sr	+ 2.49	Fe‴	+ 0.063	I	− 0.797
Ca	+ 2.28	Co	− 0.045	Br	− 1.270
Li	+ 2.369	Ni	− 0.049	Cl	− 1.694
Mg	+ 2.26	Pb	− 0.129	O	− 1.396
Al	+ 0.999	Cu	− 0.606	H	− 0.277

It may be recalled, however, that in moulds we have a form of protoplasm remarkably resistant to acids, and more sensitive to alkalies, the fatal dose for penicillium[1] spores being $\frac{n}{16}$ HCl and $\frac{n}{40}$ KOH. Again, it may happen, as with pepsin, that the alkali permanently destroys the ferment substance by causing its chemical decomposition, in the case of both pepsin and hæmatin, by splitting chlorine from it. The low inhibiting power of acetic acid is undoubtedly to be correlated with its slight dissociation.

Neilson and Brown[2] have recently shown that the anions of salts have a stimulating action on the catalytic decomposition of hydrogen peroxide by platinum black; while the cations have an inhibitory action. Cole[3] has reached the same conclusion for the action of

[1] CLARK: Botanical gazette, 1899, xxviii, p. 289.

[2] NEILSON and BROWN: This journal, 1904, x, p. 225.

[3] COLE: Journal of physiology, 1903, xxx, p. 202.

salts on diastase. Cole's results, however, are capable of the opposite interpretation to that which he gives. He considers that the chlorides stimulate, because chlorine is a strong ion; while the salts of organic acids depress or stimulate less strongly, because the organic ions are weak ions. My results and those of Neilson and Brown on ferments, and Mathews's results on nerve, in which it was shown that the anions of low solution-tension act most powerfully, indicate, in my opinion, that the *anion depresses and the cation stimulates diastatic action* instead of the reverse, as Cole supposed. While chlorine is a strong ion so far as dissociation goes, it is a very weak or inert ion chemically, owing to its high solution-tension. Moreover, the stimulating action of small amounts of acids in which the hydrogen ion is preponderant clearly indicates that the positive ion in certain concentrations acts as an accelerator of diastatic action. The depressant action of the anion is also shown by the fact that the iodides depress more than the bromides or chlorides. Although sufficient data are not at hand to show this point conclusively, we believe that the facts indicate for diastase, as Neilson and Brown have found for platinum, and Cole for ptyalin, that the two ions have an opposite action, and as Mathews has shown in nerve, the power of the ion as an accelerator or depressor varies inversely with its solution-tension. If this prove to be the case, we shall have clear evidence that ions inhibit or stimulate by means of their ionic charges, — a fact which clearly confirms the electro-chemical hypothesis of the nature of fermentation and of ferments.[1]

Conclusions and Summary.

The determination of the minimum amount of salts, bases, and acids necessary to inhibit the action of malt diastase on starch shows the following relationships:

[1] Mr. McGuigan has kindly permitted me to add a foot note to his paper. His results and the considerations given in my paper in the February number of this journal show, I think, that the ferments are bodies of very high potential (ionic potential) or low solution-tension. Certainly the oxidizing ferments must give up positive charges with great ease. They should be classified, in my opinion, not by their chemical nature, but by the voltage necessary to relieve them of these charges. This voltage can be determined indirectly, and it is our intention to continue our investigations in this direction. As I pointed out in the paper referred to, the oxidative and reducing properties, and the synthetic powers of protoplasm, are susceptible of a very simple explanation from this electro-chemical point of view. — A. P. Mathews.

a. In the different salts of the same acids the inhibitory power was found to vary inversely with the solution-tension of the cation. Salts containing cations of low solution-tension — such as mercury, silver, copper, and hydrogen — inhibit powerfully. The inhibitory power of the cation appears, therefore, to be determined by the ease with which it gives up its positive charge.

b. In the different salts of the same metal, the inhibitory power was found to vary inversely with the solution-tension of the anion. Salts of ions, such as the hydrate (oxygen), and iodide of low solution-tension (high ionic potential) inhibit more powerfully than the chlorides with a high tension.

c. The inhibitory power of the cations is also inversely proportional to the heat of ionization.

d. The inhibiting power of any salt is inversely proportional to the sum of the solution-tensions of its ions, or to the decomposition-tension of the salts.

This work was carried on under the direction of Professor A. P. Mathews, to whom I am indebted for suggestions and criticisms.

THE ALLOXURIC BASES IN ASEPTIC FEVERS.[1]

By ARTHUR R. MANDEL.

[*From the Chemical Laboratory of New York University and Bellevue Hospital Medical College.*]

THE chief constituents of the cell nucleus are nucleoproteids which on cleavage yield a class of basic products called alloxuric bases, nuclein bases, xanthin bases, or, as suggested by Emil Fischer, purin bases. Burian and Schur,[2] in their work on the relationship of alloxuric bodies to human metabolism, found, incidentally, that whenever hypoxanthin, xanthin, uric acid, thymus, or nucleic acid were injected, or introduced per os, into the human body or into a dog a rise of temperature invariably followed.

On the other hand, it is well known that the number of leucocytes in the blood is very markedly increased in cases having any surgical lesion. This leucocytosis should lead to an increased elimination of the alloxuric bodies. I therefore thought it desirable to determine the uric acid and alloxuric bases in surgical fevers. Cases were selected in which strict antiseptic precautions had prevented infection.

The amount of alloxuric bases and uric acid was determined by the method suggested by Salkowski. Where possible, the determinations were made in duplicate and the average results given. In all cases examined the diet was eight ounces of milk every two hours. The food ingested had therefore no influence on the elimination of alloxuric bases. The twenty-four hours urine was taken before the operation and for six to seven days after the operation.

The temperature was taken by the rectum every three hours and the average for twenty-four hours is given in the results.

The leucocyte count was made daily at two P. M. The following analytical tables and accompanying schematic diagrams show the temperature, number of leucocytes, uric acid and purin bases observed in three typical cases.

[1] This thesis was awarded the William T. Lusk Memorial Science Prize in 1902.

[2] BURIAN and SCHUR: Archiv für die gesammte Physiologie, 1901, lxxxvii, p. 239.

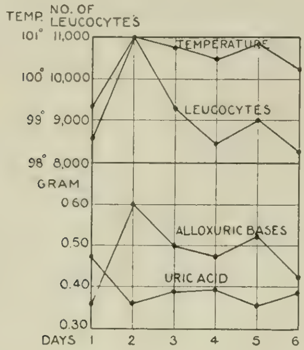

FIGURE 1. — Case J. H. (hernia). Primary union, no infection, true aseptic fever.

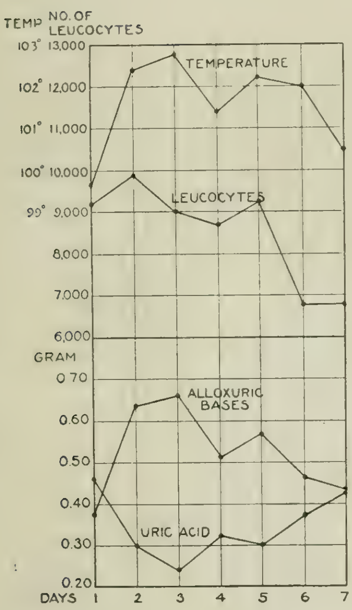

FIGURE 2. — Case J. A. Resection of knee joint for tubercular arthritis.

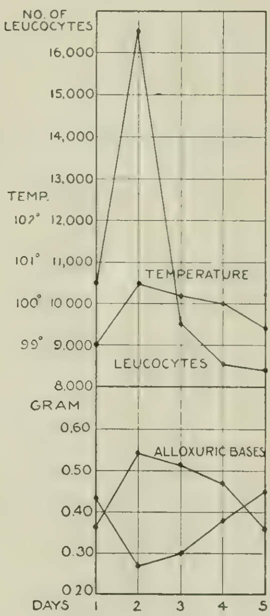

FIGURE 3. — Case M. S. Resection of hip joint after almost complete recovery from tuberculous arthritis. Primary union.

It will be seen that there is a very striking relationship between the quantity of alloxuric bases eliminated in the urine, and the average temperature observed at the same time.

TABLE I.

Case J. H. (hernia). Primary union, no infection, true aseptic fever.

Date.	Av. 24-hr. temp.	Leucocyte count.	Alloxuric bases.	Uric acid.	Total nitrogen.	Remarks.
			grams	grams	grams	
24 hrs. before operation	98.9°	9,400	0.038	0.47	12.33	Diet: 8 oz. milk q. 2 h.
1st day after	101.0°	11,000	0.060	0 35	12.24	"
2d " "	100.7°	9,200	0.050	0.38	12.77	"
3d " "	100.4°	8,600	0.048	0.39	12.99	"
4th " "	100.8°	9,000	0.051	0.35	13.24	"
5th " "	100.2°	8,500	0.042	0.38	13.46	"

TABLE II.

Case J. A. Resection of knee joint for tubercular arthritis.

Date.	Av. 24-hr. temp.	Leucocyte count.	Alloxuric bases.	Uric acid.	Total nitrogen.	Remarks.
			grams	grams	grams	
24 hrs. before operation	99.8°	9,200	0.039	0.46	12.36	Diet: 8 oz. milk q. 2 h.
1st day after	102.2°	9,800	0.061	0 30	12.07	"
2d " "	102 6°	9,000	0.064	0.24	12 28	"
3d " "	101.4°	8,800	0 051	0.31	12.49	"
4th " "	102.2°	9,200	0.055	0.30	13.42	"
5th " "	102.0°	6,800	0.047	0.37	14.02	"
6th " "	100.6°	6,800	0.044	0 42	14.48	"

The quantity of uric acid eliminated varies with the alloxuric bases, that is, the alloxuric bases increase in amount as the quantity of uric acid diminishes, and when the uric acid rises the alloxuric bases fall.

The pronounced coincidence between the temperature and the alloxuric bases eliminated led me to make experiments to prove that

alloxuric bases, when introduced into the blood, cause a rise in temperature. A female monkey weighing 2.6 kilograms was kept on a diet of bananas, and the rectal temperature taken every two

TABLE III.

Case M. S. Resection of hip joint for deformity, after almost complete recovery from tuberculous arthritis. Primary union.

Date.	Av. 24-hr. temp.	Leucocyte count.	Alloxuric bases.	Uric acid.	Total nitrogen.	Remarks.
			grams	grams	grams	
24 hrs. before operation	99.0°	10,600	0.038	0.41	13.44	Diet: 8 oz. milk q. 2 h.
1st day after	100.4°	16,400	0.056	0.26	7.05	"
2d " "	100.2°	9,400	0.053	0.31	9.37	"
3d " "	100.0°	8,500	0.049	0.40	8.98	"
4th " "	99.6°	8,400	0.039	0.44	11.29	"

hours. After obtaining the average temperature, four milligrams xanthin dissolved in two cubic centimetres of two per cent sodium carbonate solution, was injected subcutaneously, and an immediate rise in temperature was observed. Sodium carbonate alone produced no such effect.

The following temperature chart shows this very plainly:

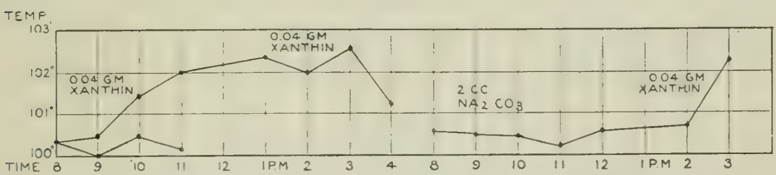

FIGURE 4. — Monkey, 2.6 kilos. Temperature curve after the injection of 0.04 gm. xanthin.

To prove further the influence of the alloxuric bases or their derivatives on temperature, I drank a strong decoction of coffee, after having obtained my average temperature by a number of hourly determinations. As is known, caffein of coffee is 1-3-7 tri-methyl xanthin, and if xanthin or its derivatives are temperature-raising substances, we would expect a rise in temperature on the administration of large quantities of coffee.

My average temperature for the day, on a constant and mixed diet, was 98.20 F. by mouth, and on taking a decoction of sixty grams of

coffee, my temperature rose to 100.3°, remained at this point for several hours, and then fell to normal.

The diagram given below shows the temperature and leucocyte count, as well as alloxuric bases and uric acid eliminated at the same time. As would be expected, no increase in leucocytes was observed.

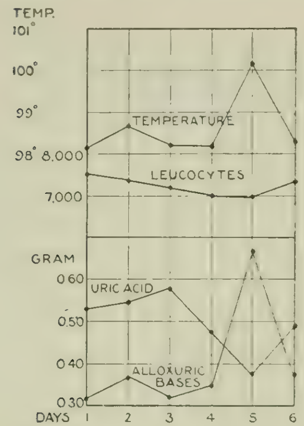

FIGURE 5.— Case A. M. Given decoction of 60 gms. coffee.

These observations indicate that in fevers there is a distinct relation between the rise of temperature and the appearance of certain incomplete products of cell oxidation, as shown by the excretion of the alloxuric bodies. The supposition that these bodies are directly concerned in the production of fevers is strengthened by the fact that the administration of xanthin and caffein produces a decided rise in body temperature in otherwise healthy individuals. It is of course at present an open question whether to consider the alloxuric bases as the sole cause of the febrile temperature or not, but it seems safe to assume that if other substances are concerned in this process, they are undoubtedly of the same origin and similar nature, *i. e.* intermediary products of cell metabolism. Their source must evidently be looked for in the circulating leucocy tesor the fixed lymphoid tissue, and also probably in other tissue cells.

TABLE IV (REFERRING TO FIG. 5).

Case A. M. given decoction of 60 grams coffee.

Date.	Av. 24 hr. temp.	Leucocyte count.	Alloxuric bases.	Uric acid.	Total nitrogen.	Remarks.
			grams	grams	grams	
1st	98.3°	7,400	0.031	0.53	11.41	Regular diet
2d	98.7°	7,300	0.036	0.55	10.56	Regular diet and 350 grams thymus
3d	98.3°	7,200	0.031	0.58	9.52	Regular diet
4th	98.4°	7,100	0.035	0.48	9.83	Regular diet
5th	100.3°	7,000	0.065	0 37	8.32	Regular diet and decoction of 60 grams coffee
6th	98.4°	7,200	0.037	0.48	10.61	Regular diet

In this connection it is interesting to note that individuals, for instance, children with good reaction ability and high leucocytosis, usually also show rather high febrile temperatures.

One other point is of especial interest in connection with these experiments. In all our cases the output of uric acid is decreased in direct proportion to the increase of alloxuric bases. This can only be due to lessened oxidation of these substances to uric acid, and it attracts attention in view of the fact that oxidation is usually considered increased in febrile processes. This interesting phenomenon may be thus explained. We may assume that in a number of pathological conditions the ability of the cells of the organism to oxidize certain substances is directly interfered with. Probably this applies to the so-called aseptic fevers, as, for instance, rise of temperature after severe operations, anæsthesia, convulsions, poisons, cachexias of various forms. In all these disturbances we may suppose that temporary or more permanent injuries to the cells inhibit their oxidation ability for a shorter or longer period; the consequences will necessarily be lessened, incomplete oxidation of substances to their proper end-products.

It has been noted by von Jacksch[1] that the purin bodies in the urine of tuberculous patients may increase from a normal equivalent of 4.4 per cent of the total nitrogen excreted, to one representing 11.3 per cent, or even 17.39 per cent. This indicates abnormal cell destruction, and my work would suggest that the liberated purin bases may have had decided influence on the febrile temperatures observed. It is unfortunate that von Jacksch has not reported temperature findings in cases he examined.

It would be a most interesting task to repeat my observations with regard to other products of metabolism in different forms of febrile conditions, with especial reference to the immediate cause of the fever.

But whatever may be found by further investigations, it seems well-established by my experiments that incomplete products of tissue metabolism, such as the alloxuric bodies, are important factors in the production of the febrile temperature.

I am indebted to Dr. B. Farquhar Curtis for suggesting this work.

[1] Von Jacksch: Zeitschrift für klinische Medicin, 1902, xlvii, p. 1.

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